Methods of Development of New Anticancer Drugs

U.S.A.--U.S.S.R. Monograph

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METHODS OF DEVELOPMENT OF NEW ANTICANCER DRUGS

National Cancer Institute Monograph 45

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Consulting Editors:
Joseph F. Saunders
Stephen K. Carter

Technical Editor:
Florence I. Gregoric

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Foreword

Frank J. Rauscher, Jr.

During the historic Moscow Summit Meeting of 1972 between the former President of the United States, Richard M. Nixon, and Premier Leonid I. Brezhnev of the Soviet Union, eleven agreements were culminated for American–Soviet cooperation in science and technology. Among these was the Agreement for Cooperation in the Fields of Medical Science and Public Health signed on 23 May 1972 by the former U.S. Secretary of State, William P. Rogers, and the U.S.S.R. Deputy Minister of Health, Dimitri D. Venediktov. The problem areas to be pursued jointly included, initially, cardiovascular and oncologic diseases and the effects of the environment on human health.

Approximately one month following the Nixon-Brezhnev Summit, a delegation of American cancer chemotherapists proceeded to Moscow for the first Joint U.S.A.–U.S.S.R. Meeting on the Chemotherapy of Malignant Neoplasms. During the one-week session, the cancer specialists and scientists of both countries agreed to specific aspects of cooperation including the exchange of anticancer drugs for preclinical and clinical evaluation; the exchange of scientists and information on cancer chemotherapy; the convening of joint meetings for discussion of specific topics, problems, and questions on the chemotherapy of diverse types of neoplasms; and the joint publication of the results of collaborative research on questions dealing with cancer chemotherapy.

It was obvious during these initial exchanges that there was a major effort in both countries toward the discovery and development of antineoplastic drugs. The scientists acknowledged that years of experience existed in both nations in the synthesis of new chemical structures and their evaluation for anticancer activity in a wide range of experimental systems. Extensive pharmacologic and toxicologic testing procedures had been developed in both countries, and there was evidence of a long history of interaction with the regulatory agencies of each nation for ultimate approval of new drugs for clinical trial. Each nation had worked extensively on clinical trial methodology, and both had accrued data on a wide variety of compounds that had gone through the entire evaluation system and, subsequently, had demonstrated their clinical value in the cancer patient.

Because of this mutual experience, the American and Soviet delegations were convinced that the initial publication evolving from the U.S.A. and U.S.S.R. cancer chemotherapy effort should be a joint monograph on anticancer drug development in both countries. The scope of the monograph would be to detail the anticancer drug development programs of the U.S.A. and the U.S.S.R. and, ultimately, to serve as a valuable reference for those scientists throughout the world who are involved in this important area of biomedical science.

At the outset, it was recognized that the compilation of material and preparation for publication of an extensive monograph jointly by the scientists of both countries would not be a simple task. The problems of geographic separation, language differences, and translation from one language to the other were superimposed upon the usual exigencies of two different scientific groups collaborating in a joint publication. Thus the completion of this monograph has taken several years, but the investment of time and effort was indeed worthwhile because of the international importance of the product.

I would be remiss if I did not highlight other aspects of the United States–U.S.S.R. cancer chemotherapy program that were moving forward vigorously while this monograph was in preparation. Much of the information contained in this document was generated in the course of these efforts. First, there has been an exchange of 134 drugs and chemicals between the two countries, 49 from the U.S.A. and 85 from the U.S.S.R. Of the 49 American compounds, 34 are in clinical use or trial, 6 are in preclinical development, and 9 are chemicals under evaluation for possible anticancer activity. Seventeen of the Soviet preparations are in clinical use or preclinical development, and the remainder are being subjected to biologic testing. The American drugs CCNU, hexamethylmelamine, DTIC, and streptozotocin have been studied extensively in the Soviet Union, whereas in the United States, detailed investigations have been undertaken on the clinical efficacy of the Soviet drugs ftorafur, asaley, and fluorodopan.

Both countries have accumulated a large body of data on each other's drugs in preclinical studies and are using the different testing systems developed by each side. The results of this effort have set a stage for correlation of test systems for anticancer drug screening, as well as a bank of information for another monograph to be published jointly.

Perhaps most significant was the exchange of scientists which afforded them the opportunity for understanding the different techniques being utilized in both countries for preclinical and clinical studies. During such exchanges, there too was the evolution of experimental studies to be undertaken such as the joint protocol for therapy of bronchogenic carcinoma. Discussions during such less formal exchanges revealed mutual compatible approaches to the treatment of this disease in both nations.

Thus this monograph serves as an illustration of a significant, tangible product of American–Soviet cooperation on the problem of malignant neoplasia. Progress of this nature reaffirms the need for and the value of American–Soviet collaboration, not only together, but with the nations of the world in the struggle against cancer.

Frank J. Rauscher, Jr., Ph.D.
Director, National Cancer Program

PART I

Drug Development in the United States and U.S.S.R.: Commentary on the Cooperative Accomplishments

C. Gordon Zubrod[1] *and* Nikolai N. Blokhin[2]

The Agreement between the United States and the U.S.S.R. on health matters is in its fifth year. The subagreement, or protocol, in the chemotherapy of cancer was reached on June 30, 1972. Before this concord, the exchanges between the two countries had been slight and sporadic, and joint efforts in health investigations did not exist. The agreement not only permitted mutual observance of each other's program, but also quickly led to practical exchanges in certain areas in which the two programs had common interests.

The progress made under this protocol has been sufficiently substantial to warrant publication of this joint monograph that offers a full description of both programs in chemotherapy and sets down all the methodologies in current use. Such descriptions should benefit not only the scientists of the two nations but chemotherapists generally.

During the first meeting of the U.S.–U.S.S.R. missions in June 1972, it became apparent that there were many differences in the organization of chemotherapy research in the two countries. The major difference was the vertical structure of Soviet experimentation, which is built around relatively autonomous research institutes, and the horizontal structure of investigative work in the United States, with a large Federal effort at the National Cancer Institute (NCI) and collaboration through grants and contracts with many of the universities and research institutes. The problem in this first meeting was to assess the two programs and locate the areas of sufficient similarity to permit easy collaboration, without worrying about the many differences.

The meeting was successful in reaching agreement on areas of cooperation (See appendix I for text protocol), which included exchange of information, scientists, and clinically useful drugs, the publication of this monograph, and the initiation of a collaborative, concurrent clinical trial. Although not part of the formal protocol of understanding, it was agreed to examine the feasibility of exchange of some drugs that were still in preclinical development.

PROBLEMS OF COMMUNICATION AND EXCHANGE

It must be recognized that this agreement represented the best judgment of the participants as to what might be feasible. The next 18 months were spent in defining and overcoming some of the obstacles to effective implementation. Chief among these were the problems of communication; the language difference was the most immediate challenge. Fortunately, Dr. Oleg Selawry of NCI, and a number of Soviet scientists, especially Nikolai Blokhin, Nikolai N. Trapeznikov, Natalia Perevodchikova, and August Garin, of the Institute of Experimental and Clinical Oncology, U.S.S.R. Academy of Medical Sciences (IECO),[3] were fluent in both languages. This fortuitous circumstance allowed rapid progress, because the various scientific and medical problems could be discussed with the depth of understanding required. It seems highly desirable for future collaborative efforts that both sides be represented by bilingual scientists.

Another difficulty imposed by language differences was the translation of lengthy documents containing all the preclinical and clinical data needed for clearances of drugs by the respective drug regulatory agencies, i.e., the Food and Drug Administration of the United States and the Pharmaceutical Committee of the U.S.S.R. Each worked out its own solution, but the translation still constitutes a source of some delay because of the extent of the data.

At first there were additional prolonged delays in information exchange because of the unfamiliarity with each other's routes of communication. By trial and error, the shortest critical paths were eventually located. The delays disappeared after Secretary Weinburger and Dr. Petrovsky arranged the installation of a Telex in October 1973.

Many other solutions to the problems of exchange and communication were worked out during 1973 by scientists visiting the other country after the initial mission. While each visitor contributed to the refinement of these arrangements, we wish in particular to mention the visits of Drs. Trapeznikov, Garin, Perevodchikova, Syrkin, Holland, and Selawry. In general, we have been impressed by the rate of progress that can be achieved during the relatively longer visits when a scientist spends most of his time at IECO or NCI. Probably, this progress relates to the deeper understanding reached during a longer stay and the mutual respect and confidence thus engendered. We also recognize that rate of progress is impeded by the language barrier.

Finally, the appointment of Dr. Joseph Saunders of NCI and Dr. Yuri Puchkov of IECO as permanent administrators for the program has sharply reduced the problems of communication and exchange.

COMMENTARY

There is no need to list all the accomplishments that will appear in the papers that follow. A few comments may be made. The exchange of scientists has been satisfactory, but we recommend that in the future there should be an increased proportion of longer visits, during which the scientist would be working in a single institution for most of his visit. The exchange of information is now performed rapidly and effectively, although attention should be given to the problems arising from reporting clinical data from the trials under way. The exchange of clinically active drugs has proceeded well. The NCI has had difficulty in arousing clinical interest in additional alkylating agents and, in the future, would prefer to exchange other types of antitumor drugs. Of particular significance has been the exchange of adriamycin and its analogues for pre-

[1] Formerly, Director, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014. Currently, Director, Comprehensive Cancer Center of Florida, University of Miami School of Medicine, Miami, Fla. 33124.

[2] Director, Cancer Research Center, Academy of Medical Sciences, U.S.S.R., Moscow, U.S.S.R.

[3] Now known as the Cancer Research Center.

clinical study. The possibilities of this most important new agent, by finding better congeners, will be increased by this exchange.

CONCLUSION

Although the initial steps in implementation seemed slow, there has been rapid acceleration, and progress has been more extensive than anticipated. As a result, discussion of expanded goals seems in order, and there are opportunities to cooperate in the whole area of cancer treatment rather than in the single modality of chemotherapy. Indeed, the need for the expanded role has been highlighted in the discussions on the joint activities on the lung cancer protocol.

Historical Background of the National Cancer Institute's Drug Development Thrust

C. Gordon Zubrod,[1] Saul A. Schepartz,[2] *and* Stephen K. Carter[3]

Descriptions of cancer are as old as is the literature of medicine. According to Moodie (*1*), Esper of Erlangen, in 1774, described a lesion believed to be an osteosarcoma of the femur in a cave bear of the Pleistocene (glacial) period. Descriptions in Egyptian papyrus suggest cancerous lesions. The external manifestations of cancer were described by Hippocrates and his school, and their attempted classification still persists.

The 19th century saw the beginning of true experimental cancer research. In 1851, Joseph Leidy of Philadelphia reported the first experimental transplantation of a malignant tumor. Arthur Nathan Hanau, in 1889, was the first to transplant cancer in rats successfully. This technique was developed by Carl Oluf Jensen who, in 1903, succeeded in carrying a mouse cancer through 19 generations of grafts without alteration of its microscopic structure.

Since 1937, when Congress created the National Cancer Institute (NCI), the United States has been officially engaged in an assault against this disease group, which is one of man's oldest, most persistent, and most costly. Our society's attack on cancers has been enlarged periodically and has gradually gained ground. The advances and the failures of previous years, plus knowledge recently gained and tools developed, have now combined to propel a new thrust. The mandate for the enlarged effort is the National Cancer Act of 1971, a statute symbolizing a new national determination to conquer these diseases.

From the time of its creation, NCI has been the primary agency through which the United States Government has sought to marshal biomedical research toward the goal of controlling human cancer. The Institute's budget in 1938 was $400,000; its budget now exceeds $400 million. The Institute has invested its research dollars through universities and medical schools, nonprofit research organizations, commercial firms, and through its own laboratories and clinical facilities. The bulk of that investment has taken the form of research grants that provide support to biomedical scientists outside Government for investigating the nature of cancer and its cause and prevention, diagnosis, and cure.

To some observers, research progress has seemed too slow, yielding few significant reductions in the overall impact of the disease. This apparent paucity of results stems in part from the fact that cancer encompasses more than 100 clinically distinct diseases. Thus even the significant progress that has been made in several types of cancer does not seem dramatic when so many others have remained resistant to prevention and cure. Furthermore, cancer is inextricably linked to fundamental life processes that are far from being fully understood. The difficulty of the cancer problem is illustrated by the fact that, despite the progress that has been made, one of every four U.S. citizens will develop cancer. According to present trends, 665,000 new cases and nearly 350,000 deaths from cancer were expected in 1976.

The Division of Cancer Treatment (DCT) in NCI has as its long-term goal the achievement of normal life expectancy in cancer patients. Short-range objectives are to increase the number of patients responding to treatment and to prolong the period of disease-free remission.

Since 1954, NCI has been developing the means by which these objectives can be met. The core of this activity has been the establishment of a central focal point to provide leadership of the national effort aimed at achieving eradication of the disease through the use of drugs. Activities have encompassed every phase of drug development, beginning with the acquisition or synthesis of new chemical compounds through the completion of clinical trials and the introduction of active antitumor drugs into medical practice. This systematic effort on the part of the Government was undertaken to fill a need that was not being met by either universities or industry, primarily because research in this area was too costly for them to do on their own.

In 1972, DCT was given the additional responsibility of monitoring and assessing the national effort to control cancer through surgery, radiotherapy, immunotherapy, and combined modality therapy. This decision, as part of enacted legislation (National Cancer Act of 1971) and increased funding, has expanded the scope of the program to encompass all modes of treatment and to provide balance, coordination, and integration of all component activities.

DEVELOPMENT OF THE CANCER CHEMOTHERAPY PROGRAM

Cancer chemotherapy, in common with drug treatment of many chronic, acute, and infectious diseases, has its roots in early periods of recorded history. A number of remedies have survived to modern times and are, in fact, still undergoing investigation and evaluation by modern techniques.

During the early days of modern cancer research before World War II, chemotherapy investigation paralleled the development of research tools and explored basic and empiric leads available at the time. A number of experimental studies resulted from clinical observations. Coley's mixed toxins, arsenicals, colchicine, and related mitotic poisons stimulated experimental research in a number of areas. On the other hand, Warburg's classic theory, proposed in 1926 (*2*), stimulated (with little success) a number of therapeutic attempts to control cancer by the use of low-pressure oxygen (*3*) and respiratory poisons (*4*). Extensive studies on the therapeutic effectiveness of numerous dyes (*5*) resulted directly from the theories of Ehrlich.

The role of hormones in the growth and control of cancer dates back to the infancy of endocrine physiology at the end of the 19th century, with empiric observations on the effect of

[1] Formerly, Director, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014. Currently, Director, Comprehensive Cancer Center of Florida, University of Miami School of Medicine, Miami, Fla. 33124.

[2] Deputy Director for Drug Research and Development, Division of Cancer Treatment, National Cancer Institute.

[3] Formerly, Deputy Director, Division of Cancer Treatment. *Present address:* Director, Northern California Cancer Program, Palo Alto, Calif. 94304.

castration in producing regression of breast and prostate cancer in man (*6, 7*). The "rational" basis of hormone therapy did not develop until the mid-1930's with the discovery of an acid phosphatase in the spleens and kidneys of cattle and swine (*8, 9*) and its presence in large amounts in the prostates of adult humans, prostate tumors, and in the sera of patients with prostate cancer (*10*). These discoveries led to the findings of Huggins and Hodges in 1939–41 (*11*), which suggested a rational basis for the use of castration or estrogen therapy in the treatment of disseminated cancer of the prostate. The work of Huggins and Hodges may be considered the beginning of the modern era of cancer chemotherapy.

Despite the preoccupation of early cancer investigators with problems of immunology, chemical carcinogenesis, and mammalian genetics, their investigations produced many of the research tools essential to chemotherapy. Four of the more widely used transplanted tumors, sarcoma 37 (S37), sarcoma 180 (S180), Walker 256, and Ehrlich ascites tumor, were developed before 1930. The development of inbred strains of mice in the early 1920's stimulated extensive use of mammary tumors for chemotherapy studies.

In the decade before World War II, a small number of embryonic drug development programs emerged. One of these was the systematic study of aldehydes by Boyland in Great Britain in which spontaneous breast tumors of mice (*12*) were used. Technical difficulties in the acquisition of adequate supplies of mice with spontaneous tumors and the inability to confirm or extend the results obtained by Strong (*13*) with heptaldehyde led to the abandonment of the study.

Furth, in the United States, initiated studies of arsenicals in experimental leukemia, but his inability to obtain financial support forced him to discontinue the project.

Lettré set up a small program in Germany for screening chemicals (dyes and mitotic poisons) to determine their effects on tumor cells in culture. He also developed techniques for the use of the Ehrlich ascites tumor. Although this work was interrupted by the war, the screening techniques of Lettré, particularly with ascites tumors, were widely used afterward in the United States and Japan.

Shear, of the U.S. Public Health Service Office of Cancer Investigations at Harvard University (later consolidated with the Pharmacology Laboratory of the old National Institute of Health to form NCI in 1937), set up a screening program in the mid-1930's to test and isolate bacterial polysaccharides capable of eliciting the Schwartzman phenomenon and producing hemorrhage and necrosis in S37. Toward the end of World War II, this screening activity was extended to synthetic compounds and plant extracts and became the first project involving an interinstitutional cooperative effort (*14*). By the early 1950's, more than 3,000 chemicals and several hundred plant extracts had been tested; a number of these caused necrosis of the tumors. The weak link in this broadly conceived program was an inability to maintain even a limited clinical activity. As a result, the program was largely discontinued in 1953, and the loosely organized collaborative effort with other institutions disintegrated. Only two agents developed by Shear et al. received clinical evaluation when the program was stopped (the polysaccharide, which Shear and co-workers had isolated from bacteria, and α-peltatin), but a number of others were subsequently selected independently through the Chemotherapy Program and are undergoing clinical trial.

EARLY ORGANIZED PROGRAMS

World War II interrupted many activities in cancer research. However, a number of war research programs, particularly in war gases, nutrition, and antibiotics, were of major significance in establishing a firm basis for the development of organized research programs in cancer chemotherapy.

Although poison gas was not used by the United States, an active program of classified research was conducted under military auspices on a variety of war gases. Many new compounds were synthesized and tested, and considerable interest developed in those with vesicant properties. One of these (sulfur mustard) was manufactured in large quantities by both Allied and Axis powers. An accident during transport of this material exposed a number of individuals to the mustard gas, with the result of a deficiency of cell elements in their blood. The extension of these fortuitous observations to the use of a related compound, nitrogen mustard, in cancer therapy soon demonstrated that chronic leukemia and lymphomas such as Hodgkin's disease responded in a spectacular though temporary manner similar to that seen after treatment of these diseases with X-irradiation. These findings paved the way for development of a vast program in the synthesis and evaluation of alkylating agents during the next two decades.

Research continued actively in the field of nutrition in several universities, the National Institutes of Health, and some industrial laboratories, with special emphasis on the isolation and identification of folic acid, which was shown to be essential for normal blood formation and growth of certain types of tumors. Diets deficient in folic acid produced a blood and bone marrow pattern resembling that produced by the administration of nitrogen mustard or X-irradiation, but the change was easily reversed when the nutrient was given. As the war ended, pteroylglutamic acid was isolated, identified, and synthesized; interest in antivitamins led to the synthesis of antifolic compounds.

Concomitant with these developments was a series of extensive and controversial studies by Lewisohn and his associates on the regression produced by yeast extracts in spontaneous tumors. Lewisohn subsequently associated these effects with crude pteroylglutamic acid concentrates (*15*). The circumstances that led to the use of folic acid antagonists in the treatment of acute leukemia in children by Farber et al. (*16*) opened a whole new area of therapy.

A third area of war research, antibiotics, led to the establishment of mass screening programs with crude fermentation products and development of large-scale fermentation and isolation programs by the pharmaceutical industry. Although crude preparations of penicillin were reported to affect tumors, an active material could not be isolated. These efforts resulted in isolation of a number of toxic materials, such as the actinomycins, which subsequently were demonstrated to have antitumor activity.

With the stimulus provided by the war research and the work of Huggins and Hodges and Shear, the end of hostilities ushered in an era of intensified interest in cancer chemotherapy research. In addition to expansion of the NCI program into the first interinstitutional collaborative chemotherapy program (*14*) mentioned previously, broadly based drug development programs were initiated at the Sloan-Kettering Institute for Cancer Research under Rhoads, at the Chester Beatty Institute in Great Britain under Haddow, and at the University of Tokyo in Japan under Yoshida. A drug development program was established at the Children's Cancer Research Foundation under Farber after his discovery that aminopterin produced spectacular, though temporary, remissions in acute leukemia.

Although interest in chemotherapy stimulated a number of academic investigators to explore a variety of transplantable tumors as test models, most had no clinical outlets in which to

evaluate their laboratory findings in man. Only the Sloan-Kettering Institute, the Children's Cancer Research Foundation, and Columbia University College of Physicians and Surgeons could offer both laboratory and clinical facilities in the United States. In Great Britain, the Chester Beatty Institute was the only institution with both laboratory and clinical resources.

The war research in antibiotics and the achievements of Farber provided a number of pharmaceutical companies with a strong stimulus to participate actively in cancer chemotherapy programs. In the United States this interest was spearheaded by Lederle Laboratories, which established a major screening program, and by Hitchings of the Burroughs Wellcome Company. In Great Britain, a major drug development program was initiated at Imperial Chemical Industries under Rose et al. (*17*). Other pharmaceutical companies became affiliated with some of the existing screening programs.

The Sloan-Kettering Institute emerged rapidly under the dynamic leadership of Rhoads as the dominating force in cancer chemotherapy throughout the world. Rhoads' organizing genius reoriented virtually the entire program and staff of the war effort with nitrogen mustards at the Chemical Warfare Service into the chemotherapy program developing at the Sloan-Kettering Institute. He also established a standardized screening system with a well-established transplanted tumor S180 as the principal screen. This method was a calculated departure from the use of spontaneous tumors. Since S180 grew in outbred mice, a continuing supply of animals for mass screening operations was thus assured.

Rhoads sparked the interest of many of the major chemical and pharmaceutical companies in this country and abroad to submit materials to the screening program by concluding a number of formal agreements with them. They agreed to accept materials on a confidential basis and to provide screening, pharmacology, and clinical evaluation of all agents developed in the program. In addition to gaining access to a wide array of secret compounds, the Sloan-Kettering Institute also obtained substantial numbers of fermentation products, including materials developed from intensive research for broad-spectrum antibiotics. By 1955, approximately 20,000 chemicals and an undisclosed number of fermentation products had been received for testing. The Sloan-Kettering program accounted for approximately 75% of the total chemotherapy screening capacity in the United States.

As a result of the increased interest of industry in submitting compounds for screening, a situation developed in which the cancer chemotherapy screening capacity was extremely limited, and fewer "new" drugs existed than clinicians who were ready to evaluate these drugs. This circumstance led to continuing pressure from industrial, academic, laboratory, and clinical sources for an expanded program in drug screening that would meet the needs of a growing number of interested investigators. The same attitude began to take hold also among laymen, particularly in the Congress.

At this time a number of new agents became known that were capable of producing dramatic but temporary remissions, principally in leukemia and lymphomas in man, and even more dramatic effects in animals. A wide variety of agents were included, such as: 1) nitrogen mustard (mechlorethamine); 2) other alkylating agents such as triethylenephosphoramide (TEPA), thio-TEPA, busulfan, chlorambucil; 3) urethan; 4) antimetabolites: aminopterin, methotrexate, 6-mercaptopurine, 8-azaguanine; 5) antibiotics: cactinomycin, puromycin, azaserine; and 6) hormones: androgens, estrogens, corticoids.

Thus the postwar years provided the basis for the development of major leads in cancer chemotherapy. Industrial and academic groups were greatly interested in screening chemical agents. Clinical and experimental cancer chemotherapy centers were developed, and chemotherapy was established as the treatment of choice for several types of cancer.

The national program in chemotherapy was initiated in July 1953, when Congress requested NCI to explore the feasibility of an engineered, directed, extramural research program in the chemotherapy of acute leukemia. An ad hoc committee of experts under the chairmanship of Dr. Walsh McDermott was appointed to make recommendations. In late December, NCI settled upon a program philosophy of voluntary cooperation and, as the initial step, awarded a series of grants to large research institutes and medical schools for the purpose of establishing or expanding integrated cancer chemotherapy research programs.

Congressional interest continued to increase and, by July 1954, it was decided that some form of interinstitutional cooperation would be necessary. To lay the groundwork for this cooperation, a new advisory committee [the Cancer Chemotherapy Committee (CCC) of the National Advisory Cancer Council] was established under the chairmanship of Dr. Sidney Farber. Several devices for improving communication were evolved: 1) publication of *Current Research in Cancer Chemotherapy* (a newsletter for informal exchange of information), 2) initiation of a series of regional, national, and international seminars and symposia, and 3) compilation of an annotated bibliography of the world literature on cancer chemotherapy. At this time, the American Cancer Society and the Damon Runyon Memorial Fund for Cancer Research joined NCI as co-sponsors of the program and named representatives to CCC. Beginning July 1, 1954, NCI increased its grant support (for that fiscal year) in the chemotherapy area to approximately $3 million.

Although these initial steps were thought to be of great value, Congress soon envisioned a much larger and more highly integrated program. To this end, NCI undertook the following: 1) establishment of a staff to operate the program, 2) enlargement of the group of sponsors to include the Atomic Energy Commission, the Veterans Administration, and the Food and Drug Administration (FDA), 3) establishment of appropriate advisory committees, and 4) creation of a contract program for the support of research and services, initiated at the request of NCI and over which the Institute exercises continuing control.

In April 1955, the Cancer Chemotherapy National Service Center (CCNSC) was established as the staff organization responsible for the program. Since NCI was to provide the major financial support, CCNSC was made a part of the Institute at Bethesda. The Center's functions were to manage NCI's portion of the program, undertake direct responsibility for contractual research, appoint needed technical advisory panels, arrange for exchange of information, promote voluntary cooperation among scientists, and render technical and other services to cooperating scientists.

In the fiscal year beginning July 1955, the congressional appropriation was increased to $5 million. The contract program was launched, and the first major interinstitutional projects were started. The first major contracts were let to four screening centers, which operated through CCNSC and accepted chemicals and natural products submitted by interested investigators for tests against animal tumors. A policy for limited confidential handling of data was adopted to protect the rights of suppliers. A Chemistry Panel established a program of supplying chemicals to cooperating investigators for use in all phases of cancer chemotherapy research. A pharma-

cology service laboratory was set up in FDA to perform toxicologic and pharmacologic studies on carcinolytic agents. Under the guidance of a Clinical Studies Panel, a number of cooperative clinical study groups were established in approximately 40 hospitals.

In the year beginning July 1956, Congress earmarked funds for endocrinology studies, and an Endocrinology Panel was established. The first antibiotic filtrates were screened and the first contracts for synthesizing compounds were awarded. In the following years, the CCNSC program continued to expand with the first industrial research contract in fiscal year 1958 and the first industrial research contract for total drug development in fiscal year 1959.

It was most fortunate in the early development of the CCNSC program that a substantial part of the entire biostatistical resources of NCI was assigned to the development of suitable experimental models for both clinical and laboratory evaluations.

The report of Gellhorn and Hirschberg (*18*) provided a unique base for the analysis of a variety of experimental procedures and the methods for their analysis. In statistical analyses presented to the Screening Panel at its meetings of September 30 and November 22, 1955, the bases for selection of the screening procedures were established. The experimental design included a heavily weighted control group used with smaller individual test groups. In addition, the size of test groups was set at the level of variability of the specific test system. This approach tended to give equal weight to the degree of inhibition observed during the test. Thus adenocarcinoma (Ca755) was designed with 10 animals per test group to give the same reliable response as a less variable test system, such as S180, which had 6 animals per group. Designs were established to provide efficient means for testing as many as 25 drugs at one time. These designs made it possible to reduce the size of experimental groups by about 20%.

It was apparent as soon as screening began that quality control would be required to manage a large screening program. It was also apparent that the conventionally used significance tests (chi-square or *t*-test), which took only internal variations into account, were wholly inadequate for these screening procedures, and that a method of evaluation based on the historical experience of the test system had to be developed.

The CCNSC was fortunate in acquiring the assistance of Peter Armitage, from the London School of Hygiene and Tropical Medicine, as a visiting scientist in 1957. Armitage and Schneiderman (of NCI), developed some of the experimental designs that formed the basis of the initial CCNSC screening procedures (*19*). They developed the mathematical models for describing the operating characteristics of a test system and the methods for developing multiple-stage, sequential test designs that concentrated and converged testing on the most promising agents (*20*).

The development of the screening program required major unprecedented expansions in the animal production resources in the United States. In this area, the CCNSC program was the prime influence in establishing the standards and facilities so necessary for the expansion in biomedical research that took place during the decade 1955–1965.

At first, heavy emphasis was placed on the use of S180, which did not require inbred mice. Since a base production of about 2 million mice existed, the needs of the screening program were easily met. The development of a steady demand to counteract the fluctuations that academic requirements created resulted in a stabilization of prices and supplies for these animals.

The use of Ca755 and leukemia 1210 (L1210) put great demands on inbred mouse production, which was probably fewer than 250,000 animals. Expansion of animal production was facilitated by the recommendation of the Screening Panel to use first generation hybrids (F_1) for screening studies. Despite some theoretical objections, the acceptance of this recommendation by the geneticists provided the basis for the CCNSC inbred mouse-production program.

The Roscoe B. Jackson Laboratory had a major part in making the initial expansion possible. The Jackson Laboratory was able to finance privately the construction of a new building and to complete most of the construction before the winter of 1955. A grant from NCI provided funds for the necessary cages and equipment. Production of F_1 mice began in spring of 1956, and a production level of 100,000 inbred mice a year was established almost immediately.

A further expansion phase to keep pace with the inbred mouse requirements was developed by a satellite production program with three commercial laboratories. The Jackson Laboratory provided DBA/2 males and C57BL/6 females in the proper proportions to these breeders for production of the hybrids. Breeder replacement was also furnished by this Laboratory, and the concentration of the inbred breeders there ensured adequate genetic control and a uniform source of mice.

Under the leadership of J. Walter Wilson (Brown University) of the Screening Panel, a conference of mammalian geneticists, held during the fall of 1956, recommended expanding the base of mammalian genetic centers and suggested a number of modified breeding methods, such as backcrossing to a single male rather than through brother × sister matings. A concept was developed of primary and secondary (or satellite) genetic centers as a means of establishing a broad genetic base for screening. Because The Jackson Laboratory could not ensure that littermates would be free of salmonellosis, the plan for primary and secondary genetic centers was not developed further, and satellite centers also became primary centers.

In 1955, disease and quality control of laboratory animals were essentially nonexistent. At its meeting on May 24, 1956, the Screening Panel recommended that the CCNSC staff arrange for the development of suitable standards for production. The Center established a long-term contract agreement with the Institute of Laboratory Animal Resources at the National Research Council to develop minimum standards for mice. Subsequently, standards were established for other laboratory animals.

The mouse standards were developed in 1957, and an accreditation program was established by CCNSC to qualify commercial producers to supply animals to screening laboratories. These standards were adopted by other major users in the pharmaceutical industry. The standards also stimulated construction of modern animal production facilities by commercial suppliers. The increased salability and profits from improved production standards resulted in the firm establishment of commercial laboratories as suppliers of inbred mice.

RECENT APPROACHES

In the early 1960's, programs began to develop along both target and basic research lines. Targeted approaches were undertaken in the development of task forces in specific disease categories (leukemia and lymphoma), laboratory models for curability of disease that could be applied to man, and programs in clinical pharmacology. The scope of the program was extended to include areas of viral chemotherapy, radiation

modifiers, and host defenses. More basic approaches were undertaken to study, e.g., the biochemistry of tumors in humans and immune-defense modifiers.

The rapid advances made in management of acute leukemia in children by combination therapy and adjustment of dose regimen to biochemical characteristics of the drugs suggested the feasibility of organizing efforts to consolidate and extend the gains in specific disease categories. To concentrate the available resources of the Chemotherapy Program on this problem, an Acute Leukemia Task Force was organized under the leadership of Dr. C. Gordon Zubrod; it included broad representation from the Collaborative Research (formerly CCNSC) staff, cooperative clinical groups, and leaders in experimental therapeutics (Schmidt, Burchenal, Kensler, and others). This group for the first time was able to maintain a sustained and collaborative effort along broad interdisciplinary lines. This approach resulted in aggressive pursuit by all the cooperative clinical groups of new leads in the treatment of acute leukemia. In the course of a few months, a number of the cooperating experimental therapeutic laboratories (Southern Research Institute; A. D. Little, Inc.; and Microbiological Associates, Inc.) were able to make an extensive and exhaustive analysis of a large number of screening systems and to select a leukemia-oriented screen. (The comparable task undertaken in 1958 took almost 3 years to complete.) It also provided stimulus for Skipper to extend his studies on the kinetics of cell kill and to exploit laboratory models that would be suitable for attempts to cure leukemia in man. The development of this activity formed a significant basis for the proposal for a revised program.

In 1965, the CCNSC was merged with the Intramural Laboratory of Chemical Pharmacology and the Medicine Branch into the new National Chemotherapy Program under the leadership of Dr. Zubrod. This program brought together for the first time an effective and responsive mechanism for a major drug development program in the cancer field, covering the full range of drug procurement, screening, pharmacology, and clinical evaluation. Each will be described in some detail in this publication, as will the logic base of the program as described in a linear array convergence technique.

The Chemotherapy Program became part of DCT as part of the newly expanded National Cancer Program in 1973. How the Chemotherapy Program is integrated into a new combined modality thrust of DCT will be described later.

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The Linear Array

C. Gordon Zubrod,[1] Saul A. Schepartz,[2] *and* Stephen K. Carter[3]

During 1966, a concerted effort was undertaken to summarize the management experience within the Chemotherapy Program, to picture the inherent program logic that had become clear by this time, and to develop new insights of significance for improvement of program management. A newly developed network planning and control technique, the "convergence technique," was called upon to aid this effort.

DESCRIPTION OF THE LINEAR ARRAY

Application of the convergence technique (*1*), developed by Mr. Louis M. Carrese and Dr. Carl G. Baker of the National Cancer Institute (NCI) staff, requires that the flow of operations within a research program be pictured logically as a starting point for devising new and improved approaches. Next, the component research activities are ordered by priority of importance to the program. The product of this effort is a convergence chart that shows three levels of research activity: a *linear array,* comprising that core of essential and minimum research required to achieve objectives; a *concurrent array,* comprising research that is not essential to operational success but contributes to refinements in the research process; and, finally, a *supplementary array* including research that is not essential to operations but is of significance to the understanding of relevant scientific phenomena that, if successfully elucidated, could have a major impact on program objectives. A resource flow is also included to account for the demands of men, money, materials, and facilities required to support the research activities in these three arrays.

In addition to assisting in the discrete definition of program objectives, the convergence technique has provided the Division of Cancer Treatment (DCT) Drug Development Program with at least two useful management tools. First, the concept of arrays (or program logic) has complemented the multidisciplinary approach to a complex, biomedically oriented problem. Second, since the linear, concurrent, and supplementary arrays represent descending orders of priority for research support, the validity of resource allocation patterns can be more readily assessed than would be the case in the absence of such a guide. Each activity can thus be assessed as to its relevance to stated program objectives as rationally as is permitted by the information at hand. Third, the use of arrays highlights the importance of securing appropriate information needed by management for proper assessment of program operations.

The convergence technique materially differs from otherwise similar network analysis-control methods because it does not deal with the specific time durations required for completion of events. The advance of time is merely implied by movement from left to right in the convergence chart. In view of the unpredictable nature of research, a specific accounting of time was considered inappropriate. The technique also provides an advantage of definitive decision points and explicit decision criteria not found in other network analysis approaches.

The total linear array is shown in appendix I.[4] In the array, the separate phases are logically dependent; that is, they give rise to information or materials required by succeeding phases. The program is outlined by means of three *flows,* which permit the development of new drugs and animal models that will select drugs for effectiveness and safety. As new agents emerge with estimated probabilities for effectiveness and safety, the three flows converge into clinical trials of these agents. The flows are divided into *phases,* and more finely into *steps;* the titles of the phases and steps indicate their principal objective. The steps are subdivided into groups of specific *projects.*

Of the three flows at the logical beginning of the program, one (flow 2) deals with the development of new drugs, whereas flows 1 and 3 deal with the use of knowledge about active drugs to establish animal model systems that help in the selection of the best new drugs for clinical trials.

Flows 1 and 3 use past experience with drugs that are highly active in clinical cancer to develop animal model systems that will predict the optimum therapeutic index. Knowledge from these models leads to the formation of screens that select new agents for effectiveness and safety. The output of new drug development flow feeds into these screens, and the drugs that qualify are further studied pharmacologically and in clinical trials.

The development of a logical plan for cancer chemotherapy requires that several assumptions be made, and it is essential that they be stated clearly. These assumptions underlie any major drug development program, but it is most likely that the following are valid for the DCT Cancer Chemotherapy Program:

1) New drugs with possible antitumor effectiveness should be sought both empirically and rationally from the widest possible base among synthetic chemicals and natural products.

2) Drugs can be found with sufficient capacity to kill tumor cells and produce permanent, complete remission within the limits of acceptable morbidity to the patient.

3) Both tumor-cell kill capacity and toxic effects of drugs in patients can be predicted from drug effects in animal models.

4) Animal models based on tumor-cell kinetics can predict drug effects in patients with a higher probability of success than is possible with nonkinetic models.

5) Drug effects in vivo can be assessed adequately only when the exposure time of cells to specific amounts of drug can be established.

6) Animal models having predicted known effective drugs will predict new effective drugs; additional animal models will also be needed.

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[1] Formerly, Director, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014. Currently, Director, Comprehensive Cancer Center of Florida, University of Miami School of Medicine, Miami, Fla. 33124.

[2] Deputy Director for Drug Research and Development, Division of Cancer Treatment, National Cancer Institute.

[3] Formerly, Deputy Director, Division of Cancer Treatment. *Present address:* Director, Northern California Cancer Program, Palo Alto, Calif. 94304.

[4] This represents the linear array as originally developed. Although many specific changes have occurred since that time, the underlying logic is essentially the same.

THE LINEAR ARRAY IN THE MANAGEMENT OF DRUG DEVELOPMENT

The linear array is applied in the management of drug development in a concise way. As indicated, it is divided into evaluation phases or stages. Each stage included a series of research activities seeking to answer definitely a question believed to be a key to continued interest in a given agent, such as: Does the agent show in vivo activity? Can it be prepared in a clinically acceptable form? Does it demonstrate any insolvable toxicity problems?

The Linear Array Decision Network Group (LADNG), established within the Chemotherapy Program to apply the array to program management and to continue to do so in the expanded program, is composed of the Director of DCT and his senior scientific staff. They meet at approximately monthly intervals to consider, with the aid of brief summaries of research results, whether individual agents merit advance through the linear array. Because approval for advance is given in single stage increments, the research completed in the current stage must be evaluated favorably by the group if an agent is to advance. To assist the evaluation of research, technical subcommittees, which have a filtering function, have been appointed to review data and develop the summary judgments that are transmitted to the senior group. For example, while a working-level committee reviews the initial screening data for all agents entering the system, LADNG is advised of only those agents that meet the basic criteria for in vivo activity. The senior group reviews these recommendations and determines which of the agents will be allowed to enter the next stage of testing, accomplished either by NCI's scientists in NCI facilities or by scientists working under contract to the DCT Program.

The group considers each decision unit or criteria test in the order in which it appears in the array for each compound showing activity in initial screening. At each point several possibilities face the decisionmakers: 1) to affirm or deny that satisfactory accomplishment of a primary requirement has been demonstrated, 2) to affirm or deny satisfaction of a primary demand or accomplishment of a suitable alternative demand, and 3) to recognize the need for further testing to allow an evaluation. These decision alternatives permit 1) advancing an agent to a further stage of testing, 2) removing an agent from consideration, or 3) recycling an agent through a specific research phase to allow more definite evaluation.

Generally, it is true that serial progression through the drug development array involves progressively increasing resource expenditure per stage. For example, formulation studies are more costly than initial screening, and clinical trials in humans are more costly than preclinical toxicologic tests in large animals. Thus it is important that the program be precise in evaluating whether an agent merits entry into a subsequent stage. The test of merit that the linear array imposes provides assurance that an agent meets the criteria before it is allowed to make further demands on program resources.

CONTRIBUTION OF THE LINEAR ARRAY TO ACCELERATED PROGRESS IN DRUG DEVELOPMENT

The linear array has been used as an effective planning aid and as the primary operational control in the Drug Research and Development Program of DCT for the past 5 years. It has improved the efficiency of operations through:

1) *Increased selectivity.*—Over the past several years, there has been a definite improvement in the ability of the managers of the program to eliminate, at progressively earlier points in the processing cycle, active agents that present difficulties, such as the need for a dose form that can be satisfactorily administered, irreversible or uncontrollable toxicity, and lack of clear advantage over existing clinically tried and proved drugs.

2) *Lower processing cost.*—With the definition and explicit statement of the essential flow process and related evaluation criteria, nonessential test operations previously supported have been eliminated. As a result, the cost of such testing has been eliminated through insight provided by the linear array. Further cost reductions were attained as a result of the closer processing scheduling enabled by more detailed knowledge of flow interrelationships.

3) *Improved utilization of senior scientific staff.*—The linear array has provided a means of improving the use of senior scientific staff within the program. Much of the time-consuming subjective discussion that characterized evaluations of agents has been reduced or eliminated as a result of explicit statement of processing demands and evaluation criteria. This exposure, which provides an explicit basis for rationality, has allowed delegation of responsibility for completion and reporting on research events to lower staff levels. Similarly, as the information format and contents needs of LADNG have become better defined, the group has dealt with decisions more expeditiously. This savings in time has been attested to in statements by members of the senior groups, e.g., "I never would have believed that we could deal with as many agents in a single meeting."

4) *Process improvement.*—Although the planning sessions that produced the linear array contributed a number of suggestions for improvements of the drug development logic sequence, an even greater amount of new insight concerning how the process should be structured has developed as a result of implementation of the array. In some cases, the enhanced understanding of the overall sequence has led to implementation of some *added* testing at early stages. This testing was done when it became apparent that data (of importance in a later stage or needed in the evaluation of a later stage) could be conveniently acquired at an early point. These data were found to be necessary especially for the formulation studies. Often, the need for data became apparent too late, i.e., only after a significant, unrecoverable investment had been made. The array was also useful in highlighting interfaces when coordination was essential between groups participating in the evaluation of an agent, e.g., in contact between laboratory and clinical investigators.

5) *Increased overall organizational concern for program efficiency.*—Whereas little or no overt pressure has been placed upon operating-level scientists to organize and orient their work further along the most logical and efficient lines (beyond that which is naturally a part of their professional duties), such pressure has existed, brought about through good example. That is, as the merit of the linear array and its related logical approach have become well known, scientists within the program have been prompted to seek to organize their own work better. As a result, sublinear arrays have been developed and applied by operating-level scientists to assist detailed research within their respective operating units.

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Selection of Agents for the Tumor Screen of Potential New Antineoplastic Drugs

Harry B. Wood, Jr.[1]

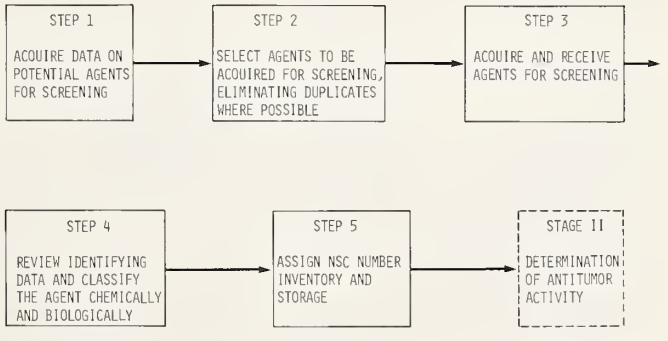
Because cancer is not one disease but a number of diseases affecting the various organs and systems of the body, many different approaches to cancer treatment may be adopted. The major approaches include surgery, radiation, chemotherapy, immunotherapy, or a combination of the four. This paper deals with chemotherapy and the search for new chemotherapeutic agents to be used singly or in combination. Research in drug development includes selection and procurement of agents for testing, bioassay of the agent in tumor systems, preclinical pharmacologic and toxicologic study, and clinical evaluations. The foundation of any drug program is the selection and procurement of agents, i.e., the selection of agents for the biologic tumor screen. To date, approximately 275,000 compounds with known structures and a similar number of natural product extracts have been supplied to the antitumor screen through the National Cancer Institute (NCI), Division of Cancer Treatment (DCT), Drug Development Branch. This discussion briefly covers the following areas: 1) the method of compound choice, 2) the chemical structures of clinically effective agents, 3) input to the screen during 1975 and a review of the results, and 4) analysis of contributions from the synthesis, plant, and fermentation areas.

When the Cancer Chemotherapy National Service Center was established in the mid-1950's, the planners recognized the need to involve the pharmaceutical industry and the scientific community at large in the program against cancer. For the pharmaceutical industry, a Commercial Discreet Agreement was drawn up that provides two fundamental safeguards: 1) the proprietary rights for compounds shall remain with the supplier company, and 2) confidentiality is observed for the materials and their sources. For the scientific community, assistance via grants in medicinal chemistry is provided to the academic and research institutes. These grants are awarded on the basis of both scientific merit and health (cancer) relevance. The Commercial Discreet Agreement with industry and the research grants to the academic community provide the cornerstone for a steady supply of all types of agents to be tested. The fact that some 20 years later we receive compounds from approximately 3,400 suppliers reflects the wisdom of early planners. One might ask, "Where do such samples originate?" Today our samples come from pharmaceutical and chemical industries, universities, research institutes, Government agencies, and our own laboratories and contract program. These sources are both domestic and foreign. Approximately 256 new suppliers were added during 1975, and the list continues to grow.

The selection and acquisition of agents for screening is depicted in The Linear Array Convergence Plan (*1*) in text-figure 1, which highlights the step-by-step development of new agents.

Step 1 provides for the development of rationales for the preselection of compounds to be evaluated and the surveillance of the literature for existing potential new drugs. Step 2 provides the basic criteria for selection: 1) from antitumor structure–activity relationships, new and promising structural classes emphasized and adequately studied classes or nonproductive structural classes deemphasized; 2) new structures or analogues developed through chemical, biochemical, cell-cycle kinetics, pharmacologic, and pharmaceutical rationales; 3) biologic activity demonstrated in other systems and non-NCI programs; 4) current and potential sources for natural products; 5) nature and extent of random synthetic compound inventory and input; and 6) submission of agents to NCI for screening without specific solicitation. Step 3 provides for the actual acquisition of the materials, taking into account: 1) the quantity desired, 2) physical–chemical constants, 3) chemical structure and identification, and 4) supporting information, i.e., source, lot number, solubility, stability, and biologic activity. Step 4 provides for the data verification and the classification of the agent chemically and biologically and identifies and eliminates duplicate materials. Step 5 provides for the assignment of the permanent National Service Center (NSC) number, inventory amount, and storage. The materials are then sent for biologic screening and evaluation, which is stage II.

Many suppliers have submitted compounds for exploratory purposes to ascertain whether their new compounds possessed any activity. Such random submissions have provided active materials; however, the percentage of active materials by this route is much lower than that submitted on a specific rationale. Random input, which includes most materials from natural products, provides the greatest variety of new chemical classes or structures. Materials developed by rational approaches are generally extensions of existing active agents or analogues. The percentage of active material developed via the rational approach will be much higher. Whereas the rational method is preferred, the exploratory approach may serve a useful purpose in providing new structural classes of compounds for testing. Unfortunately, the state of the art has not reached the



TEXT-FIGURE 1.—Linear Array Convergence Plan: Stage 1, for the selection and acquisition of agents for screening.

[1] Chief, Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014.

degree of sophistication that allows for a completely rational approach to drug design.

The development of drugs by rational design may encompass many considerations. The design of a new drug is usually determined by the specific metabolic target one seeks to affect. In this case, the proposed agent is patterned on the chemical structure of a metabolite that participates in the target cellular activity, and close structural similarity is desirable to retain the same specificity that characterizes the parent molecule. When available, knowledge of the mechanism of biochemical action of a compound in the cell and the causes of cytotoxic selectivity are used by the medicinal chemist in drug design. Drug transport or bioavailability must be considered, because the drug must reach the target site in sufficient quantity to be an effective agent. It must not be biotransformed to an inactive material enroute to the target. The binding, by means of tight covalent or weak hydrogen bonds, to RNA or DNA may be of concern. Likewise, the toxicity of the drug or its metabolites must be minimized. Stability and solubility characteristics of the drug before and during administration must also be taken into account. Availability from an economical or time factor aspect may affect its selection among several closely related drugs. Because structure–activity relationships may be important in drug development, we must qualitatively and quantitatively examine the biologic changes that result from a given structural modification. Furthermore, we must endeavor to derive from this evaluation, criteria that can serve as guidelines for the synthesis of other, possibly more site-specific, target-selective, and less toxic drugs. As we refocus our attention from the fast-growing to the slow-growing tumors, the knowledge of cell-cycle kinetics and tumor-cell age phenomena will become increasingly more important. Factors affecting metastasis and drug resistance must not be overlooked.

The clinically effective compounds officially recognized by NCI (compiled by Dr. Stephen K. Carter, former Deputy Director, DCT, NCI), as shown in table 1, fall into six broad categories: 1) alkylating agents, 2) antibiotics, 3) antimetabolites, 4) hormonal agents, 5) mitotic inhibitors, and 6) miscellaneous structures.

The chemical structures of these clinically effective drugs are shown in text-figure 2, and their sources and major activity, if any, in the animal tumor screens are listed in table 2.

In reviewing these compounds, one point to stress is the wide variety and diversity of chemical structures. Those compounds obtained from natural sources and many synthetic compounds fall into the category of exploratory input materials. The observation that many of these compounds act by different biochemical mechanisms is certainly intriguing and challenging to chemists, biologists, pharmacologists, and clinicians. The wide range of chemical structures is the basis for a continuing interest in the exploration of many new types and sources of compounds and certainly supports continued surveillance of new compounds until more sophisticated rationales are developed for drug design.

The materials accessioned and the corresponding activity data accumulated in 1975 are shown in table 3. Of 9,807 crude extracts submitted, approximately 589 active ones were found. There were 21,295 crystalline materials evaluated, and most of these were derived from synthetic sources. Among the confirmed active crystalline materials were 29 fermentation products, 8 animal and 30 plant products, and 692 synthetics. One would conclude that synthetic products were the richest source of confirmed active drugs during this period, whether by random or rational input. During 1975, 18 compounds passed decision point 2 to become candidates for preclinical toxicology, and 4 compounds (1 fermentation product, 1 plant product, 2 synthetics) progressed to initial clinical study, as indicated by the filing of an Investigational New Drug (IND) application with the Food and Drug Administration.

Where do the materials entering the program originate? Table 4 shows the number of new crystalline materials entering the program from foreign and domestic sources during 1975. Nonindustrial suppliers, including universities, research institutes, and non-NCI government sources accounted for approximately 18.8% of the materials. During the same period, the industrial community provided 73.0% of all materials, whereas compounds prepared under contract accounted for only 4.9% of the total, and approximately 3.3% were purchased from commercial sources. When the data are analyzed, it is clear that the greatest number of active compounds are prepared under contract; industrial sources are second, and the nonindustrial sources and purchased compounds tie for third. This breakdown substantiates the earlier prediction that the greatest percentage of active compounds will be analogues, found by

TABLE 1.–*Official National Cancer Institute list of effective antineoplastic drugs*

Alkylating agents	Antibiotics	Antimetabolites	Hormonal agents	Mitiotic inhibitors	Miscellaneous
BCNU[1,3-bis(2-chloroethyl)-1-nitrosourea]	Dactinomycin	6-Azauridine triacetate	Adrenal cortical compounds:	Vincristine	Hydroxyurea
Busulfan	Daunorubicin	Cytosine arabinoside	Cortisone	Vinblastine	DTIC (dimethyltriazenoimidazole carboxamide)
Chlorambucil	Adriamycin	5-Fluorouracil	Hydrocortisone		Methylhydrazine sulfate
Cyclophosphamide	Mithramycin	6-Mercaptopurine	Prednisolone		Methyl-GAG [methylglyoxal bis (guanylhydrazone)]
Dibromomannitol	Streptozotocin	Methotrexate	Prednisone		
Nitrogen mustard (mechlorethamine)	Bleomycin	6-Thioguanine	Androgens:		
L-Phenylalanine mustard (melphalan)	L-Asparaginase		Fluoxymesterone		
Thio-TEPA (triethylene thiophosphoramide)			Testosterone propionate		
Triethylenemelamine			Estrogens:		
			Diethylstilbestrol		
			Ethinylestradiol		
			Other:		
			ACTH		
			Progesterone		

TABLE 2.—*Sources and in vivo activity of clinical drugs*

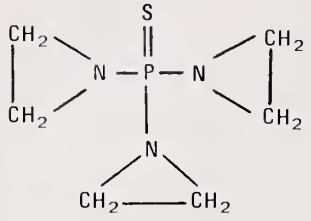
NSC number	Name	Supplier to NCI	Source	In vivo activity [a] (T/C percentages)			
				L1210	P388	B16	LL
740	Methotrexate	Lederle	Synthetic	272	>300	130	N
750	Busulfan	Burroughs Wellcome	"	N	N	N	N
752	6-Thioguanine	Francis Earle Laboratories	"	228	132	N	N
755	6-Mercaptopurine	Burroughs Wellcome	"	263	150	N	N
762	Nitrogen mustard	Merck, Sharp & Dohme	"	160	300	185	N
3053	Dactinomycin	"	Fermentation	145	>275	203	N
3070	Diethylstilbestrol	"	Synthetic	N	N	N	N
3088	Chlorambucil	Burroughs Wellcome	"	131	198	132	N
6396	Thio-TEPA	Lederle	"	180	206	N	130
8806	Melphalan	Chester Beatty	"	237	281	257	154
9166	Testosterone propionate	Syntex	Plant/synthetic	N	N	N	N
9703	Cortisone	Merck, Sharp & Dohme	"	N	N	N	N
9704	Progesterone	Syntex	"	N	N	N	N
9706	Triethylenemelamine	Lederle	Synthetic	168	271	130	N
9900	Prednisolone	Schering	Plant/synthetic	N	N	N	N
10023	Prednisone	"	"	N	N	138	N
10483	Hydrocortisone	Merck, Sharp & Dohme	"	N	N	—	—
12165	Fluoxymesterone	Upjohn	"	N	N	—	—
13875	Hexamethylmelamine	Parke-Davis	Synthetic	N	N	N	N
19893	5-Fluorouracil	Hoffmann-LaRoche	"	180	220	140	150
24559	Mithramycin	Pfizer	Fermentation	N	222	N	N
25933	ACTH	Upjohn	Animal	N	—	—	—
26271	Cyclophosphamide	Mead Johnson	Synthetic	236	>300	176	222
26980	Mitomycin C	Bristol	Fermentation	170	250	167	N
27640	5-FUDR (floxuridine)	Hoffmann-LaRoche	Synthetic	152	255	138	128
32065	Hydroxyurea	Squibb	"	278	150	N	129
45388	DTIC (dimethyltria-zenoimidazole carboxamide)	Southern Research Institute	"	160	154	145	N
49842	Vinblastine	Lilly	Plant	140	212	220	N
63878	Ara-C (cytosine arabinoside)	Upjohn	Synthetic	200	221	160	>137
67574	Vincristine	Lilly	Plant	147	242	189	N
70735	Nafoxidine hydrochloride	Upjohn	Synthetic	N	—	—	—
77213	Procarbazine	Hoffmann-LaRoche	"	152	160	132	N
79037	CCNU	Southern Research Institute	"	>300	272	300	130
82151	Daunorubicin	Farmitalia	Fermentation	166	267	>300	N
85998	Streptozotocin	Upjohn	Fermentation/synthetic	160	154	N	N
88536	Calusterone	"	Plant/synthetic	N	N	—	—
94100	Dibromomannitol	Medimpex	Synthetic	N	170	136	N
95441	Methyl-CCNU	Southern Research Institute	"	>300	190	234	>400
102816	5-Azacytidine	Upjohn	Fermentation/synthetic	>300	253	140	N
104800	1,6-Dibromodulcitol	Medimpex	Synthetic	152	130	141	N
109229	L-Asparaginase	Squibb	Fermentation	136	136	N	N
109724	Isophosphamide	Asta-Werke	Synthetic	>215	282	147	154
122819	VM-26	Sandoz	Plant/synthetic	300	250	—	—
123127	Adriamycin	Farmitalia	Fermentation	300	>300	300	N
125066	Bleomycin A	Roswell Park	"	N	150	168	158
141540	VP-16	Sandoz	Plant/synthetic	250	241	280	N
409962	BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea]	Southern Research	Synthetic	>300	>300	236	172

[a] Data from (*2*) and other data submitted to Drug Evaluation Branch, National Cancer Institute. T/C = treated/control ×100; less than 125 = inactive.

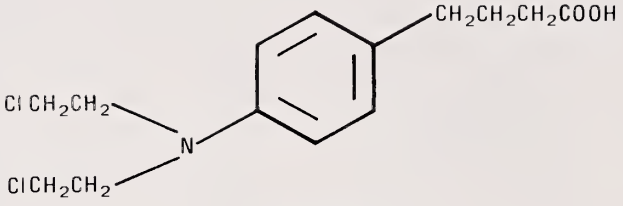
the rational approach through designed synthesis. The worldwide collection program, currently in effect for synthetics, plant and animal materials, and fermentation products, further indicates the willingness of the scientific community to participate in and endorse the cancer chemotherapy approach.

One approach to the examination of the structural classes of compounds entering the system is the analysis of ring systems (table 5). Recently, the Chemical Abstracts Service indicated that approximately 36,000 unique ring systems have been described in the literature. It is noteworthy that the Chemotherapy Program has evaluated only 4,105. A review by program statisticians indicated that, to evaluate adequately a class of compounds (e.g., one ring system), at least 200 compounds of a given structural class should be tested before it can be determined with a high degree of certainty that no active materials exist in the class.

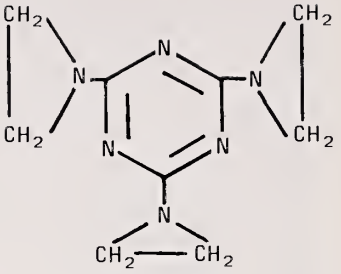
An analysis of the ring skeletons, as shown in table 5, indicates that 73 have received statistically adequate sampling. At the other extreme, there are 4,032 ring skeletons having less than 200 samples. It is certainly not the intent of the Program



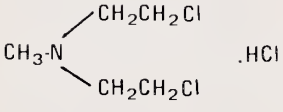
Thio-TEPA
NSC-6396



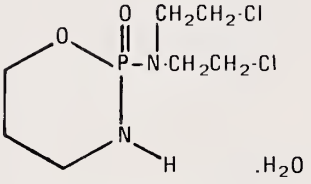
Chlorambucil
NSC-3088



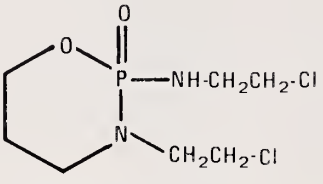
Triethylenemelamine
NSC-9706



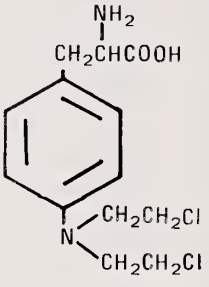
Nitrogen mustard
NSC-762



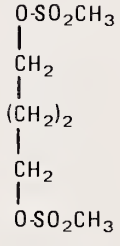
Cyclophosphamide
NSC-26271



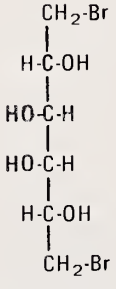
Isophosphamide
NSC-109724



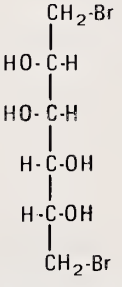
Melphalan
NSC-8806



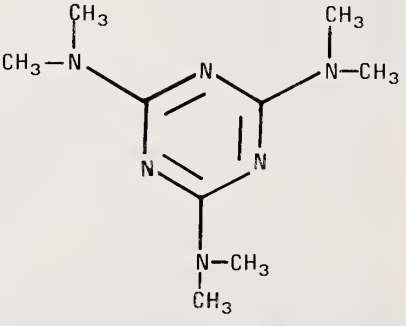
Busulfan
NSC-750



Dibromodulcitol
NSC-104800

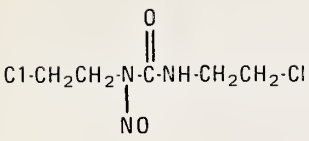


Dibromomannitol
NSC-94100

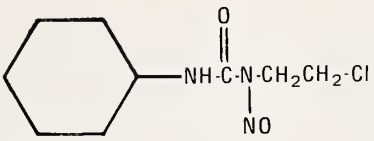


Hexamethylmelamine
NSC-13875

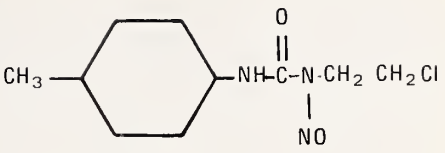
TEXT-FIGURE 2A–G.—Drugs with clinical activity.



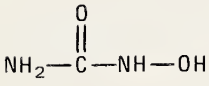
BCNU
NSC-409962



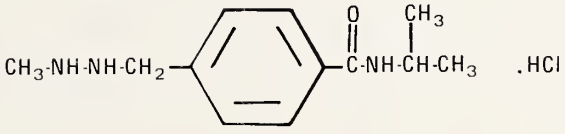
CCNU
NSC-79037



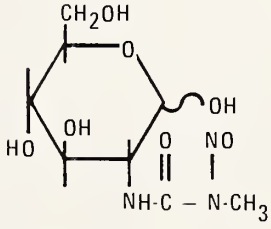
Methyl CCNU
NSC-95441



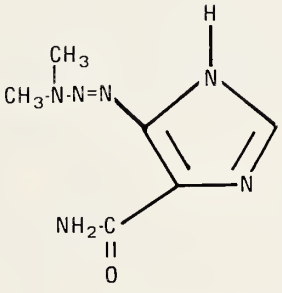
Hydroxyurea
NSC-32065



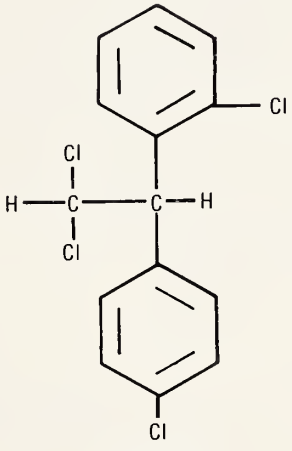
Procarbazine
NSC-77213



Streptozotocin
NSC-85998

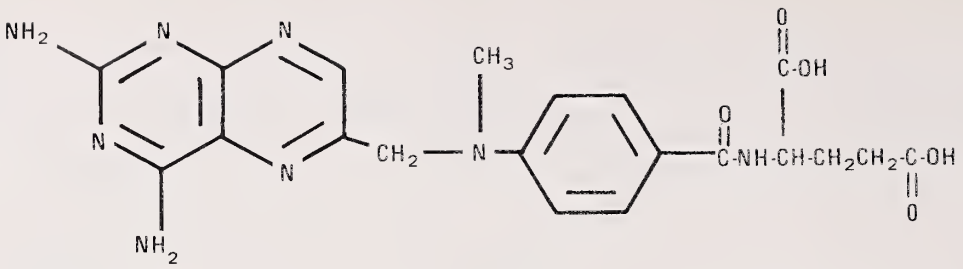


Imidazole-4-carboxamide,
5-(3,3-dimethyl-1-triazeno)
NSC-45388

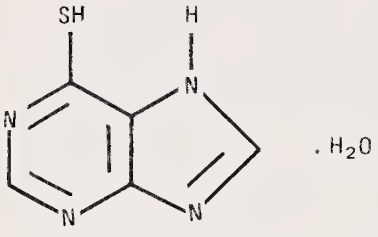


o,p'-DDD
NSC-38721

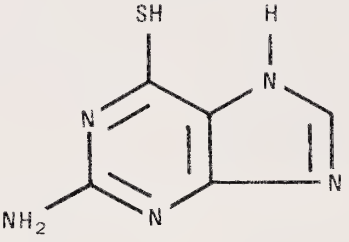
TEXT-FIGURE 2B.—Drugs with clinical activity (continued).



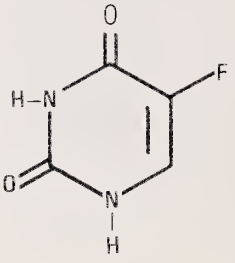
Methotrexate
NSC-740



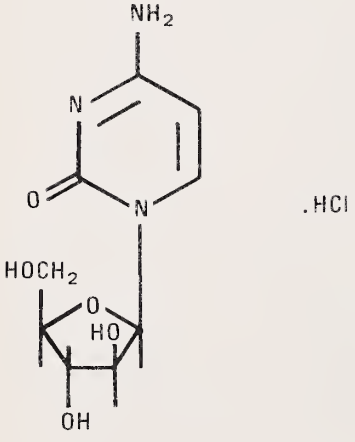
6-Mercaptopurine
NSC-755



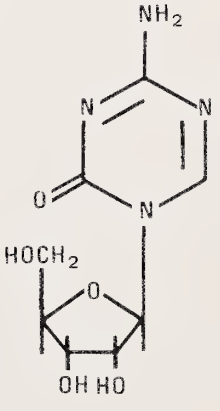
6-Thioguanine
NSC-752



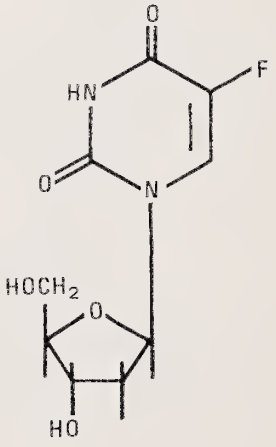
5-Fluorouracil
NSC-19893



Cytosine Arabinoside
NSC-63878

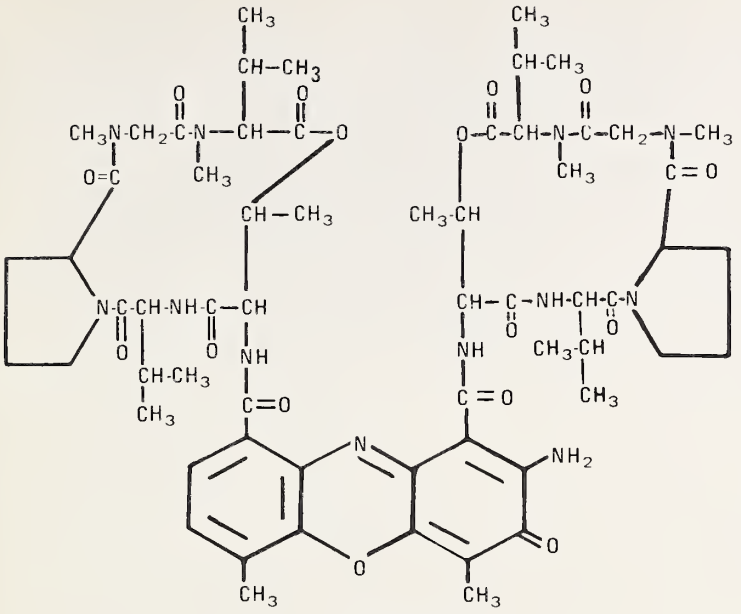


5-Azacytidine
NSC-102816

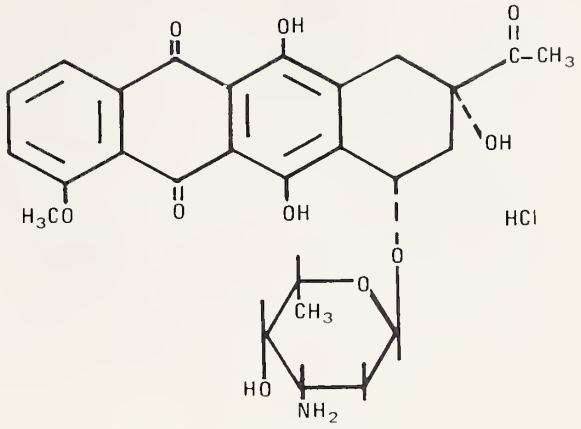


5-FUDR
NSC-27640

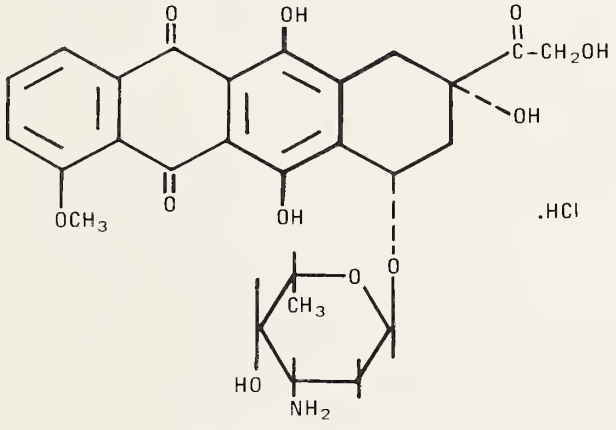
TEXT-FIGURE 2C.—Drugs with clinical activity (continued).



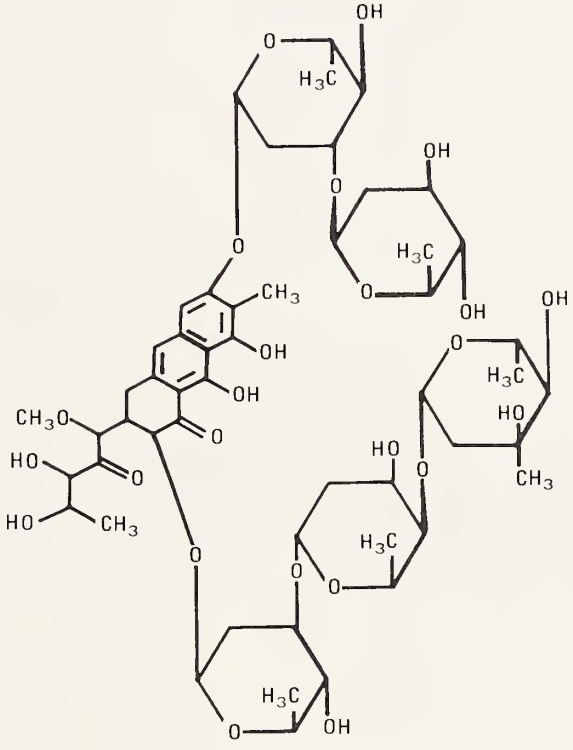
Dactinomycin
NSC-3053



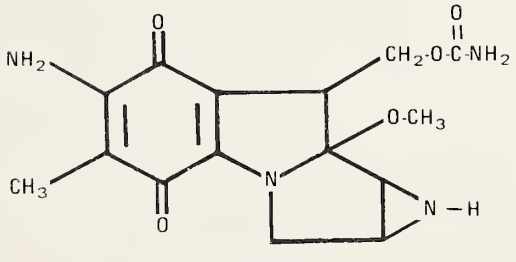
Daunorubicin
NSC-82151



Adriamycin, hydrochloride
NSC-123127



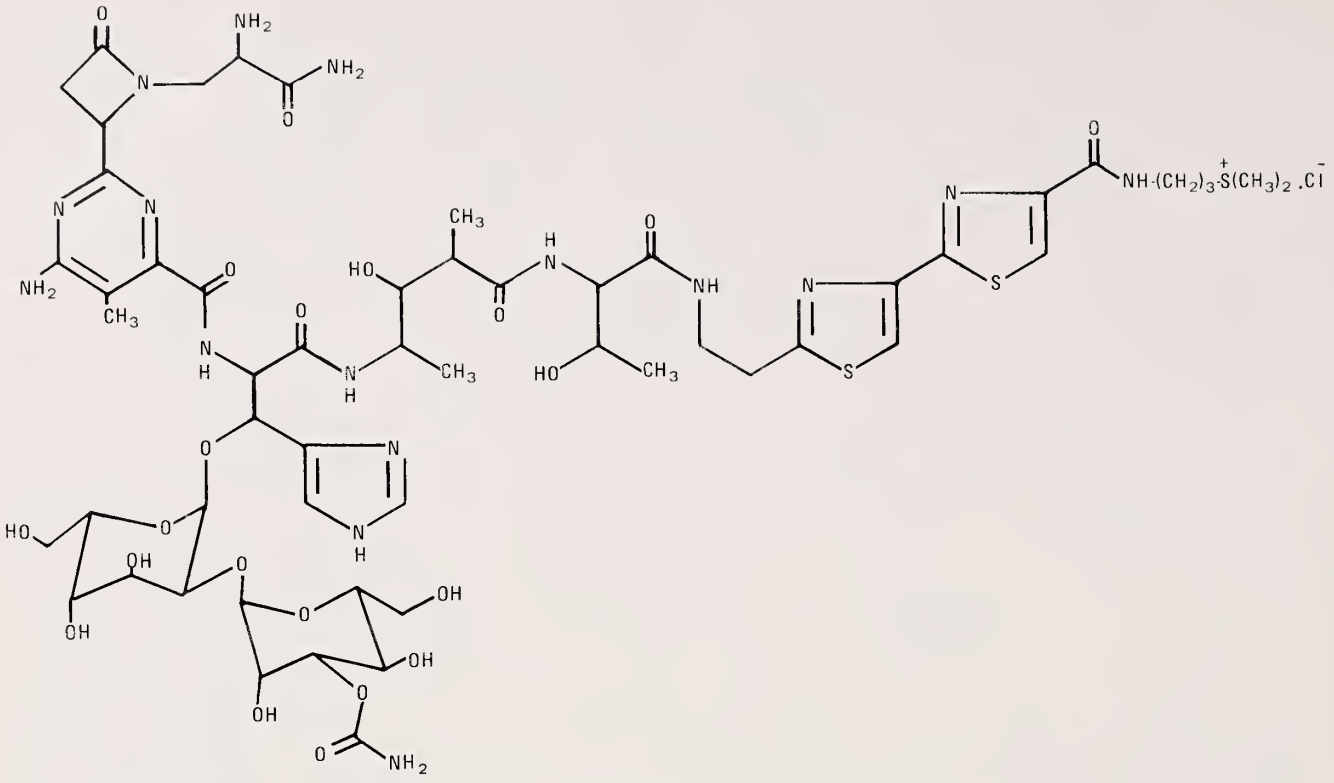
Mithramycin
NSC-24559



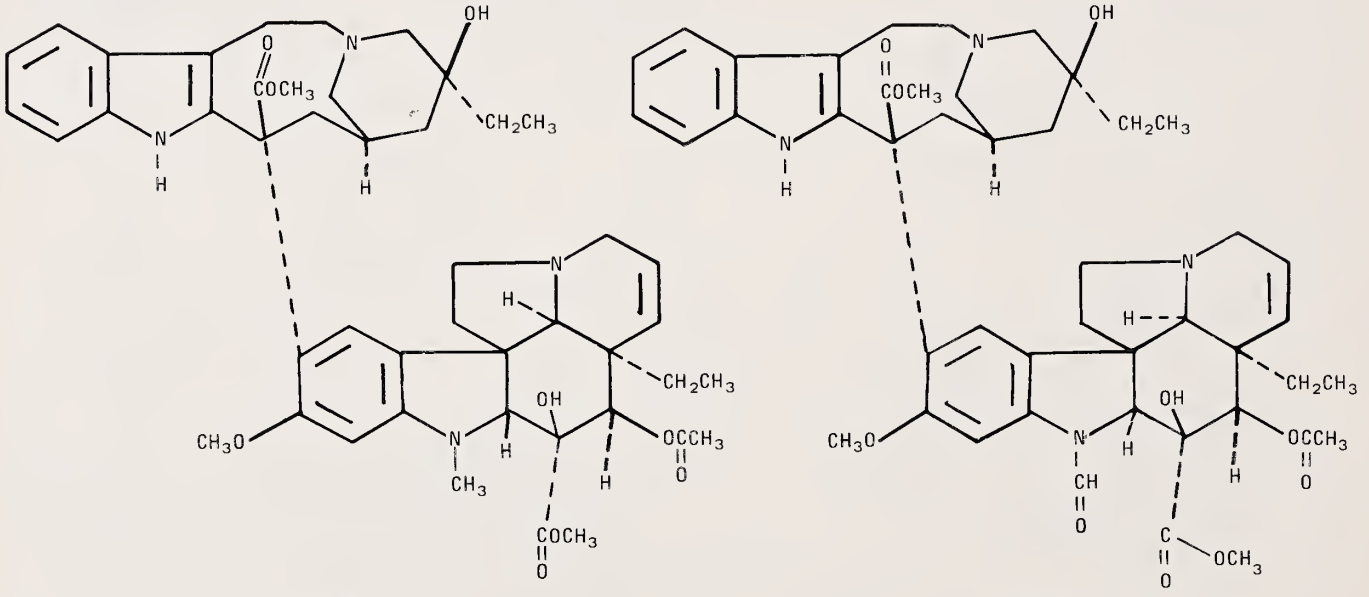
Mitomycin C
NSC-26980

L-Asparaginase
Protein (structure unknown)
NSC-109229

TEXT-FIGURE 2D.—Drugs with clinical activity (continued).



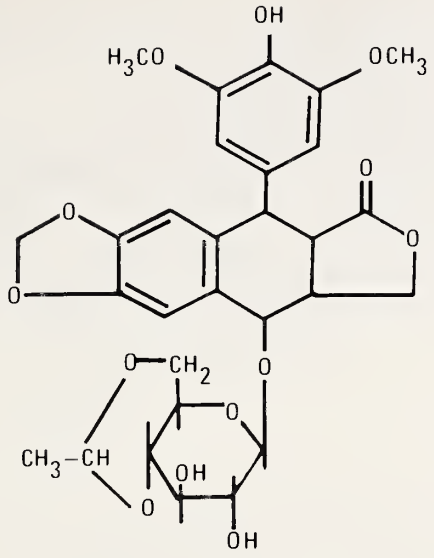
Bleomycin A_2
NSC-125066



Vinblastine
NSC-49842

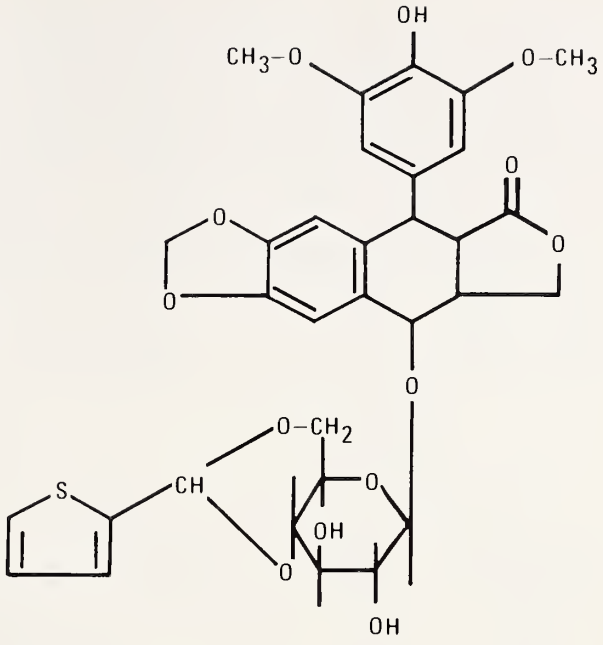
Vincristine
NSC-67574

TEXT-FIGURE 2E.—Drugs with clinical activity (continued).



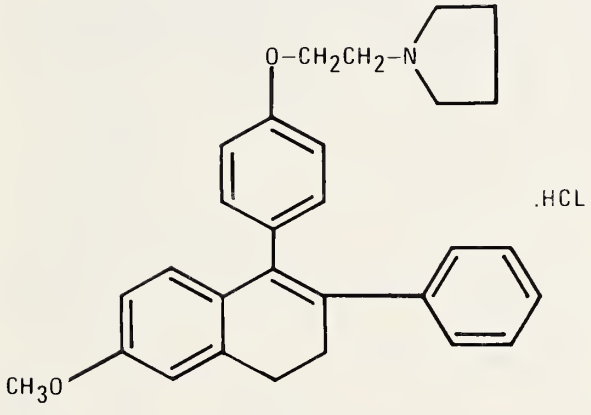
VP-16

NSC–141540



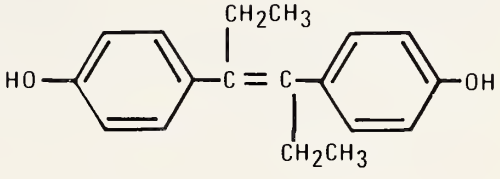
VM–26

NSC–122819



Nafoxidine hydrochloride

NSC–70735

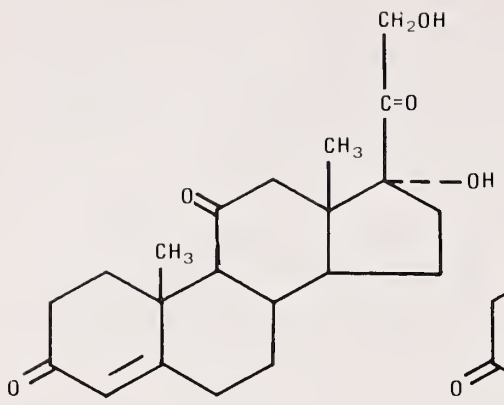


Diethylstilbestrol

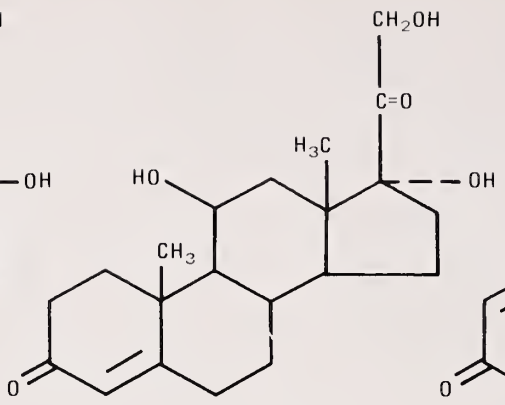
NSC–3070

ACTH
Protein (structure unknown)
NSC-25933

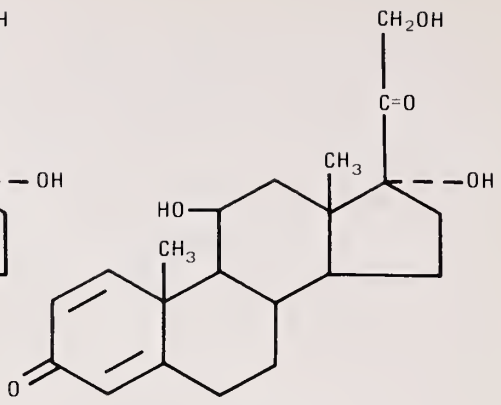
TEXT-FIGURE 2F.—Drugs with clinical activity (continued).



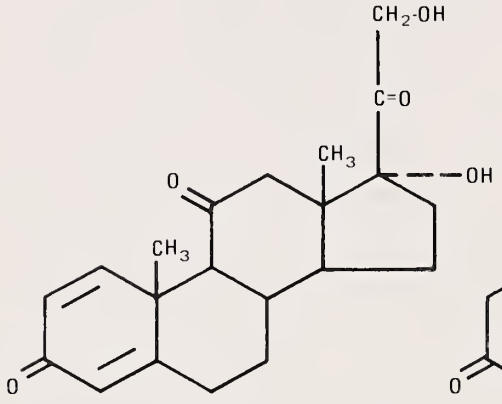
Cortisone
NSC-9703



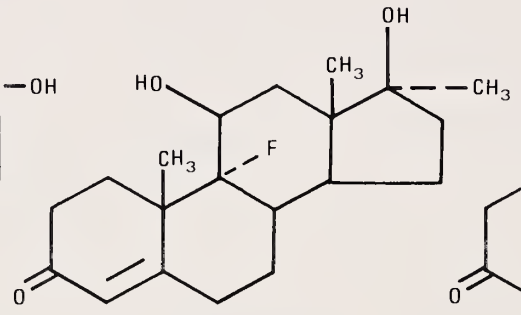
Hydrocortisone
NSC-10483



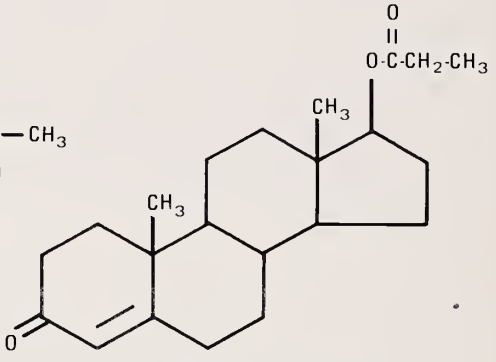
Prednisolone
NSC-9900



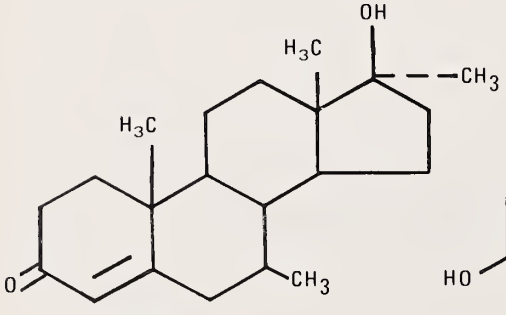
Prednisone
NSC-10023



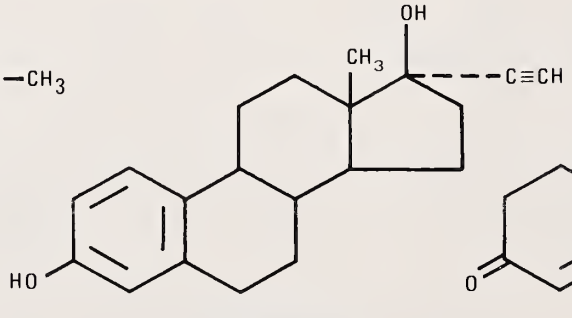
Fluoxymesterone
NSC-12165



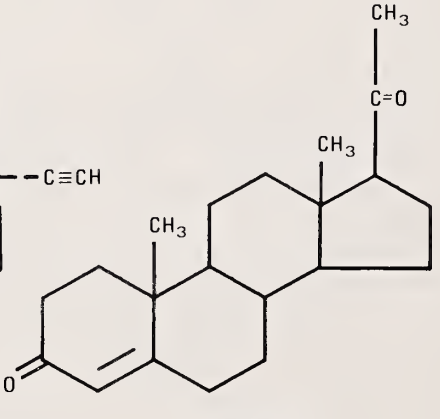
Testosterone propionate
NSC-9166



Calusterone
NSC-88536



Ethinylestradiol
NSC-10973



Progesterone
NSC-9704

TEXT-FIGURE 2G.—Drugs with clinical activity (continued).

TABLE 3.—*Materials accessioned and activity report: 1975*

Material	Crude extracts		Crystalline materials		Passed decision point 2[a]	IND filed
	Submitted	Active	Submitted	Active		
Fermentation products	1,328	141	159	29	3	1
Animal products	2,086	102	38	8	1	—
Plant products	6,393	346[b]	155	30	1	1
Synthetics	—	—	20,943	692	13	2
Total	9,807	589	21,295	759	18	4

[a] Selected as candidates for preclinical toxicologic evaluation and clinical trial.
[b] Individual species.

TABLE 4.—*New crystalline materials entering the program (1975)*

Source	Number of compounds	Percentage
Nonindustrial	3,942	18.8
Industrial	15,588	73.0
Contract	1,046	4.9
Purchased	719	3.3
Total	21,295	100.0

TABLE 5.—*Analysis of ring skeletons evaluated* [a]

Number of compounds/ring skeleton	Number of different ring skeletons
1–49	3,923
50–99	68
100–199	41
200–499	31
500–999	11
1,000+	31
Total ring skeletons (through NSC-210,000)	4,105

[a] Total ring skeletons reported by Chemical Abstracts Service = 36,000.

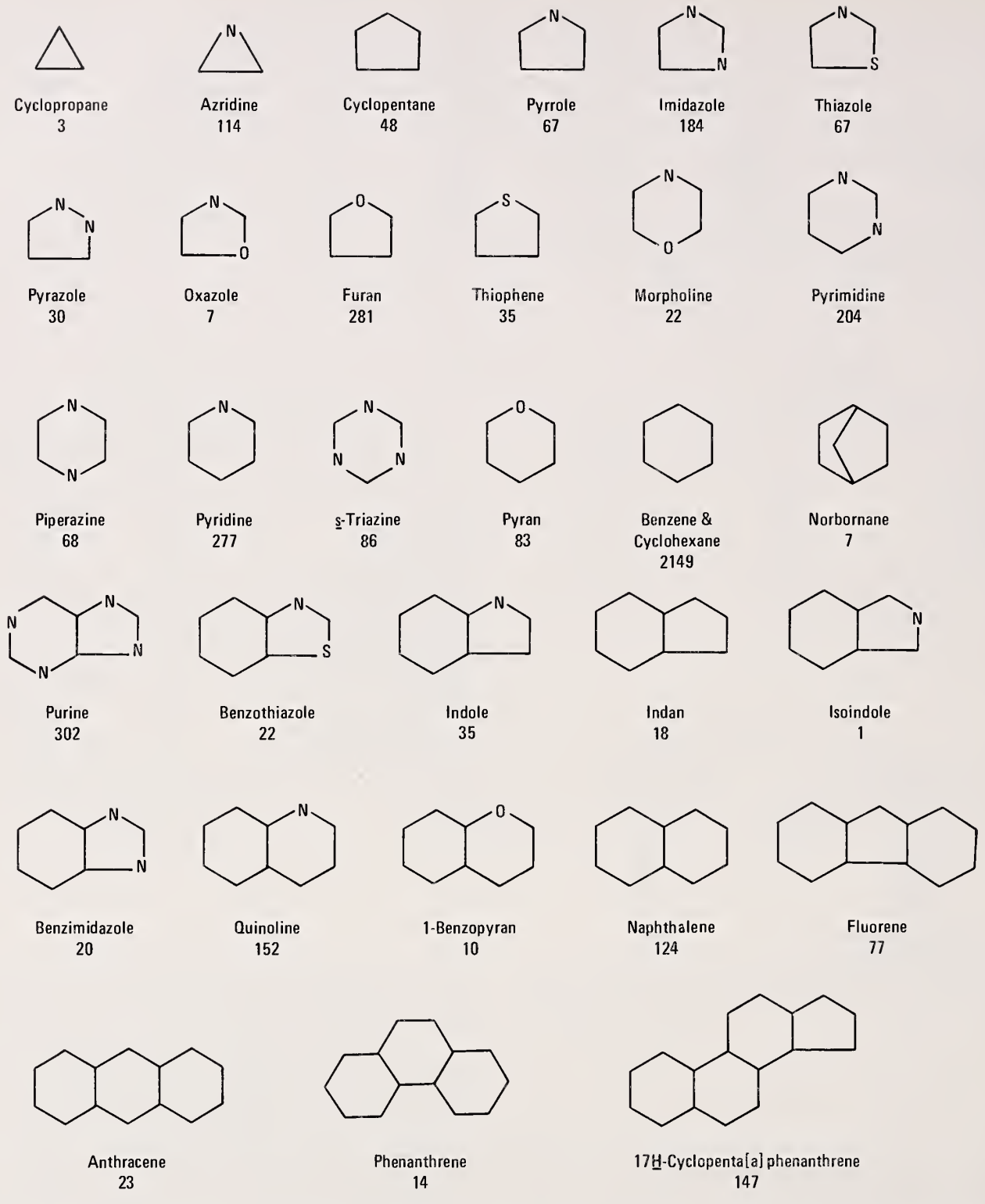
TABLE 6.—*Analysis of active rings with various atoms*

Rings containing:	Number of rings	Number of active compounds[a]
Carbon	40	2,731
Carbon-nitrogen	152	2,104
Carbon-oxygen	63	553
Carbon-sulfur	17	114
Carbon-nitrogen-oxygen	59	165
Carbon-nitrogen-sulfur	31	174
Phosphorus-other	10	21
Carbon-nitrogen-oxygen-sulfur	1	1
Carbon-sulfur-other	1	1
Carbon-nitrogen-sulfur-other	1	1
Carbon-oxygen-sulfur	6	6
No carbon	3	4
Carbon-oxygen-other	9	16
Carbon-oxygen-sulfur-other	1	1
Carbon-nitrogen-other	1	1
Total	395	5,893

[a] Some compounds contain more than one ring system.

at this time to acquire hundreds of compounds in each ring system, nor is it feasible (albeit interesting) to obtain examples of all the 36,000 ring skeletons.

Text-figure 3 depicts the ring systems in which there have been 1,000 or more examples tested in the program. For each ring system, the number shown indicates the number of active materials in that particular system. It is obvious that the ring systems with large numbers of examples are, generally, those in which activity has been observed, and this is the reason they are so well represented. For example, there have been 184 active imidazoles and 302 active purine derivatives. The benzene and cyclohexane moiety, with 2,149 actives, appears in many compounds. Text-figure 3 includes 22 ring systems having hetero atoms, i.e., ring atoms other than carbon. There are nine nonhetero compounds such as benzene and naphthalene. When the three-membered systems containing nitrogen, oxygen, and carbon (aziridine, oxirane, and cyclopropane) are compared, it is interesting that the greatest number of active compounds are found in the aziridines, or the nitrogen heterocyclic moiety. Also, when the five-membered ring systems containing one atom of nitrogen, oxygen, or sulfur (pyrrole, furan, or thiophene) and the all-carbon ring (cyclopentane) are compared, the greatest number of active compounds occur in the oxygen heterocyclic system (text-fig. 3). This circumstance probably reflects the great importance of the purine and pyrimidine nucleoside categories, which contain the furan moiety. The number of active purine and pyrimidine rings also supports the influence of the nucleosides upon the evidence of active compounds.



TEXT-FIGURE 3.—Ring skeletons with 1,000 or more examples. Numbers following names denote the number of active compounds for the ring skeleton system.

The analysis of active ring skeleton systems by atom type (table 6) shows that the total carbon ring and the carbon–nitrogen ring systems are by far the most active ring skeletons.

What are some of the reasons for preparing drug analogues? Certainly, the most important justification is to improve activity and decrease toxicity. A closely related reason might be to alter the duration of activity, producing a longer- or shorter-acting drug and thus more selectivity for a given target site. Another major motive for the preparation of drug analogues is to solve specific problems with existing drugs. For example, an improvement in the stability of a compound might decrease its metabolic destruction or decrease the excretion rate of the active component from the host. One might also be interested in altering compounds by increasing or decreasing their lipid solubility. The lipid solubility of a compound is a major factor in its ability to cross the blood–brain barrier. Certainly, drug transport is markedly affected by changes in the partition coefficient of a compound. The formulation of a drug to be given iv is directly related to its solubility in solvent systems that are suitable for human use. In addition, analogues may overcome problems of drug resistance and are useful in mechanism studies. Biochemical investigations may include studies of drug distribution, drug metabolites, enzymatic activity, and the route and rate of excretion. The use of analogues in structure–activity studies, such as the Hansch regression analysis system, should lead to a more rational approach to drug design. Economically, improved methods of production in the shortest time must also be considered.

In the area of natural products, higher plants have provided about 78,000 extracts. From these, 198 plant families show active extracts, and 64 families have yielded active crystalline compounds; some are shown in table 7. The plant product program is operated almost entirely through contract rather than voluntary submission of materials. Our contract with branches of the U.S. Department of Agriculture provides an excellent input of new materials from their worldwide collection program and their particular awareness of the time when materials should be collected. The plant parts and the weights of materials assembled are also important to our program in the event that large quantities are needed. The greatest degree of biologic reproducibility is obtained with plant material re-collected from the same source. One might wonder if all the active materials come from a single part of the plant, but in our total surveillance of plants we consider all parts, e.g., the bark, bulbs, fruit, stems, roots, and leaves. Indeed, various active substances have been found in different parts of the plant, and one must therefore evaluate as many portions as possible.

Next to the synthetic sources, fermentation products have probably supplied the greatest number of materials in our clinical program. Of the purified fermentation products isolated from the actinomycetes, fungi, bacteria, and algae, 975 have been evaluated in the Chemotherapy Program (table 8); 103 have been developed or originated by the NCI program. The largest number of active materials was isolated from actinomycetes.

In addition to the plant and fermentation products, animal extracts are being screened on a smaller scale and have shown some activity. Of approximately 2,086 animal extracts tested, 102 have shown activity.

In addition to the clinically active drugs depicted in table 2 and text-figure 2, other new structures are emerging from drug development programs at NCI and throughout the world. Some of the chemical structures of these newer drugs under development are given in text-figure 4; table 9 provides the suppliers, sources, and in vivo activities and emphasizes again the diversity of chemical structures. Simple structures, such as *cis*-diaminedichloroplatinum (NSC-119875), chlorozotocin (NSC-178248), and AT–125 (NSC-163501), and the more complex compounds, e.g., 5-methyl-tetrahydrohomofolic acid (NSC-139490), maytansine (NSC-153858), and asaley (NSC-167-780) serve to indicate that these widely varying structures are in all probability exerting their biologic activity by means of different biochemical mechanisms.

No attempt has been made to include complete reference to the vast number of publications in the field of cancer chemotherapeutic agents. However, those seeking reviews on chemical structures and natural products associated with new materials in the development of new carcinostatic agents might consider the following: Montgomery (*3–11*), Cheng and Zee-Cheng (*12–14*), Livingston and Carter (*15*), Farnsworth (*16*), Hartwell and Abbott (*17, 18*), Bloch (*19, 20*), Jewers (*21*), Goldin et al. (*22, 23*), Mead and Wood (*24*), Driscoll et al. (*25*), and Sandberg et al. (*26*).

TABLE 7.—*Plant families containing active agents of interest*

Family	Types of compounds
Aceraceae	Saponins
Apocynaceae	Alkaloids, cardenolides, sterols
Bignoniaceae	Quinones
Burseraceae	Lignans, triterpenes, sterols
Celastraceae	Large ring systems, diterpenoids
Cephalotaxceae	Alkaloids
Compositae	Sesquiterpene lactones, saponins, glycoproteins, lignins, alkaloids, flavones
Leguminosae	Proteins, saponins, rotenoids, alkaloids
Menispermaceae	Alkaloids
Nyssaceae	Alkaloids
Ranunculaceae	Alkaloids, α,β-unsaturated lactones
Rubiaceae	Alkaloids, triterpenes
Rutaceae	Alkaloids
Simaroubaceae	Alkaloids, quassinoids
Taxaceae	Alkaloids
Thymelaeaceae	Structure class unknown

TABLE 8.—*Purified fermentation products tested*

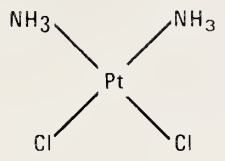
Source	Total tested	Total active
Actinomycetes	533	119
Fungi	179	23
Bacteria	42	9
Algae	5	—
Total	759	151

TABLE 9.—*Sources and in vivo activity of some unique compounds*

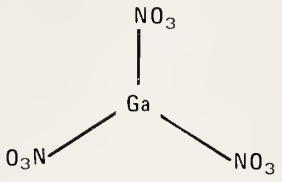
NSC number	Name	Supplier	Source	In vivo activity[a] (T/C percentages)			
				L1210	P388	B16	LL
15200	Gallium nitrate	Fort Belvoir	Synthetic	125	142	N	N
51143	2,3-Dihydro-1*H*-pyrazolo [2,3-*a*]imidazole	CIBA	"	150	N	141	144[b]
71795	Ellipticine	CSIRO, Australia	Plant product/synthetic	282	204	N	N
71851	α-TGDR	Stanford Research Institute	Synthetic	158	157	142	N
73754	Fluorodopan	Starks Associates	"	128	168	151	N
104801	Cytembena	Spofa, Czechoslovakia	"	N	169	N	N
118994	Inosine diglycoaldehyde	Wyeth	"	>300	247[b]	N	N[b]
119875	*cis*-diaminedichloroplatinum	Michigan State University	"	207	>300	279	N
125973	Taxol	Research Triangle Institute	Plant product	131	171	186	N
126849	3-Deazauridine	University of Utah	Synthetic	150	N	N	—
129943	ICRF-159	Imperial Chemical Industries	"	166	185	140	130
132313	Dianhydrogalactitol	Merck, Sharp & Dohme	"	237	282	166	N
132319	Indicine *N*-oxide	Pfizer	Plant product	140	200	153	N
135758	Piperazinedione	Merck, Sharp & Dohme	Fermentation	306	214	142	N
139105	Baker's antifol	University of California	Synthetic	N	N	N	N
139490	5-Methyl-tetrahydrohomofolic acid	Collaborative Research	"	166	127	132[b]	143[b]
141549	Acridinyl aniside	Auckland Cancer Society, New Zealand	Synthetic	200	268	278	N
141633	Homoharringtonine	U.S. Department of Agriculture	Plant product	142	300	N	N
141537	Anguidin	Bristol Laboratories	Fermentation	157	223	N	N
145668	Cyclocytidine	Terra Marine	Synthetic	>300	224	N	—
146397	Nitidine chloride	Research Triangle Institute	Plant product	141	238	N	N
148958	Ftorafur	U.S.S.R. Latvian Acad. Sci.	Synthetic	160	134	148	N
153353	L-Alanosine	Dow-Lepetit, Italy	Fermentation	182	181	N	—
153858	Maytansine	University of Virginia	Plant product	126	186	145	N
154020	Penta-aza-acenaphthylene nucleoside	University of Utah	Synthetic	169	136[b]	N	N[b]
154890	Coralyne sulfoacetate	Aldrich/Midwest Research Institute	Plant product/synthetic	140	190	N	153[b]
163063	Tripdiolide	University of Virginia	Plant product	239	150	N	N
163501	AT-125	Upjohn	Fermentation	170	215	127	N
164011	Rubidazone	Stanford Research Institute/Rhone Poulenc	Fermentation/synthetic	180	287	245	N
165563	Bruceantin	University of Virginia	Plant product	132	217	168[b]	N
167780	Asaley	IECO, U.S.S.R.[c]	Synthetic	158[b]	195[b]	N	N[b]
167781	Diiodobenzotepa	IECO, U.S.S.R.	"	176	232	148	N
176319	Quinolinium	Auckland Cancer Society, New Zealand	"	>300	247	N	N
178248	Chlorozotocin	Southern Research Institute	"	>300	203	241	N
178249	5-Methoxysterigmatocystin	Bristol	Fermentation	166	206	N	N

[a] Data from (*2*) and other data submitted to Drug Evaluation Branch, NCI. T/C= treated/control × 100; less than 125= inactive.
[b] Preliminary data.
[c] Now known as the Cancer Research Center.

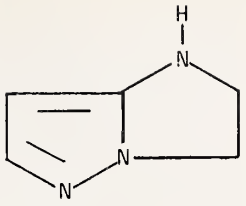
In summary, we may conclude that 1) structural requirements for activity are extremely varied; 2) the many structural differences among drugs suggest that different biochemical mechanisms may be involved; 3) many new structural entities are still available from various sources; 4) random input provides the greatest number of new materials at this time; 5) the largest percentage of active agents from both new materials and analogues are derived from a rational input; 6) contractors supply the greatest percentage of active compounds; and 7) there is every reason to believe that more active drugs with greater specificity will be discovered.



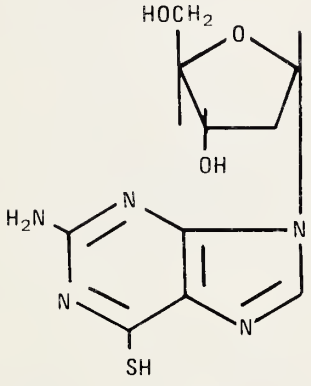
cis-Diamminedichloro-
platinum
NSC-119875



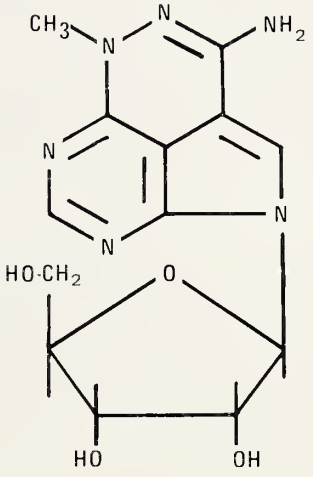
Gallium nitrate
NSC-15200



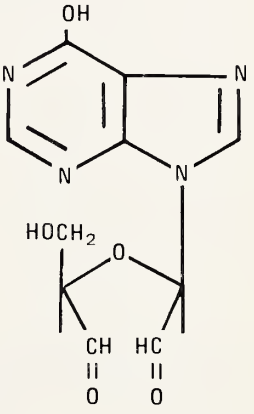
2,3-dihydro-
1H-Pyrazolo[2,3-a]imidazole
NSC-51143



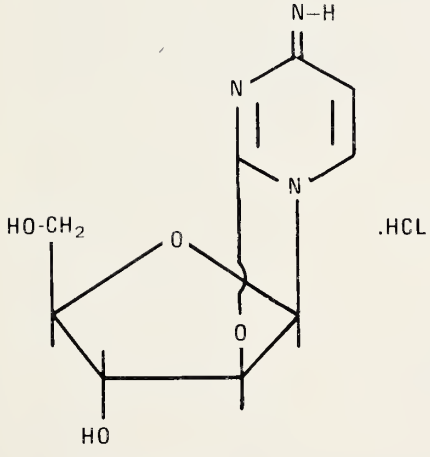
α-TGdR
NSC-71851



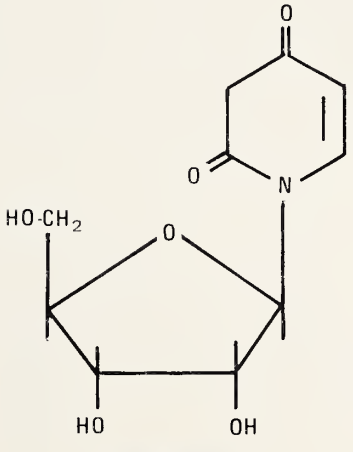
Pentaazaacenaphthylene riboside
NSC-154020



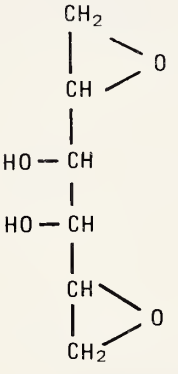
Inosine diglycolaldehyde
NSC-118994



Cyclocytidine
NSC-145668

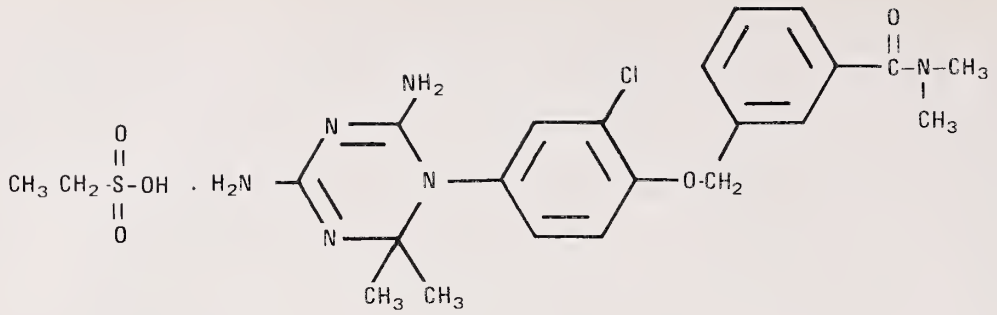


3-Deazauridine
NSC-126849

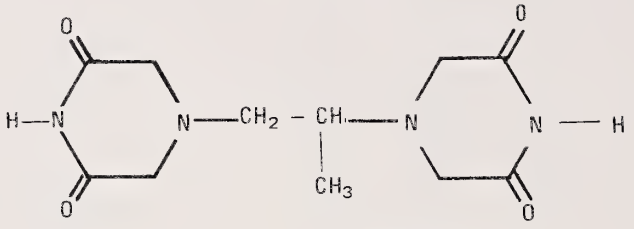


Dianhydrogalactitol
NSC-132313

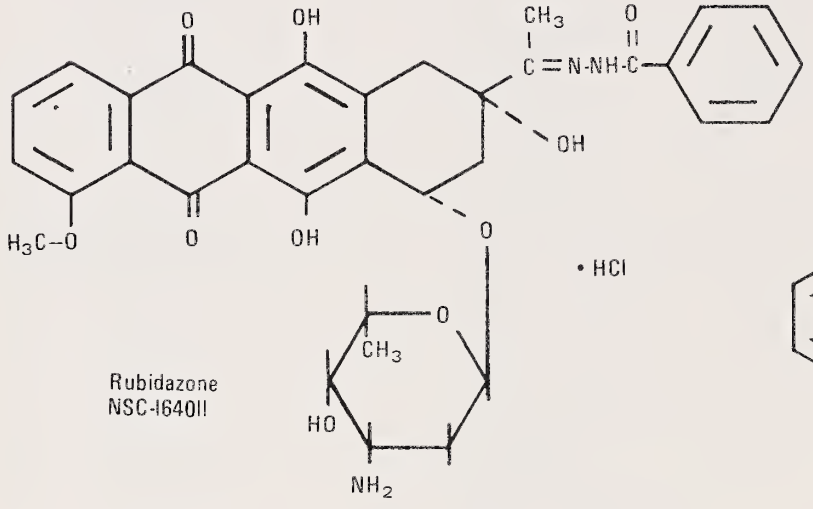
TEXT-FIGURE 4A–F.—Some unique compounds in development.



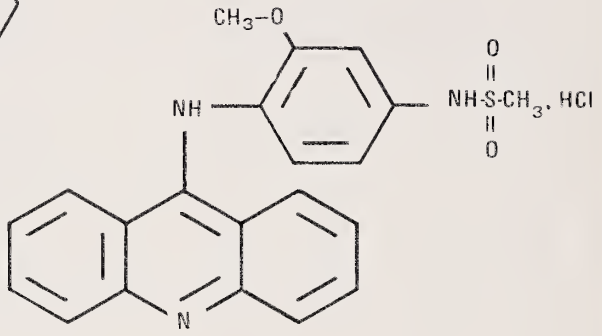
Baker's antifol
NSC-139105



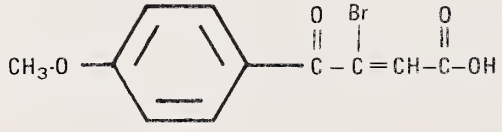
ICRF 159
NSC-129943



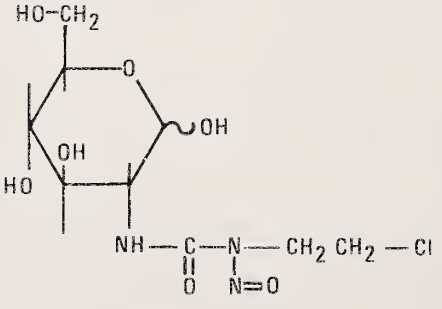
Rubidazone
NSC-164011



4'-(9-Acridinylamino) methanesulfon-m-anisidide
NSC-141549

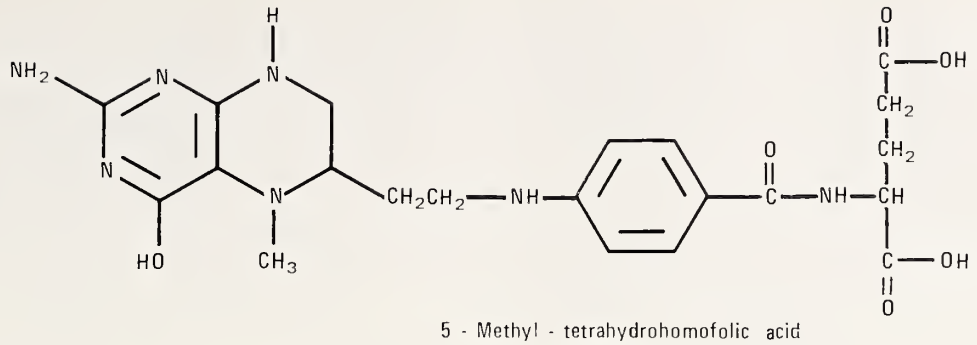


Cytembena
NSC-104801

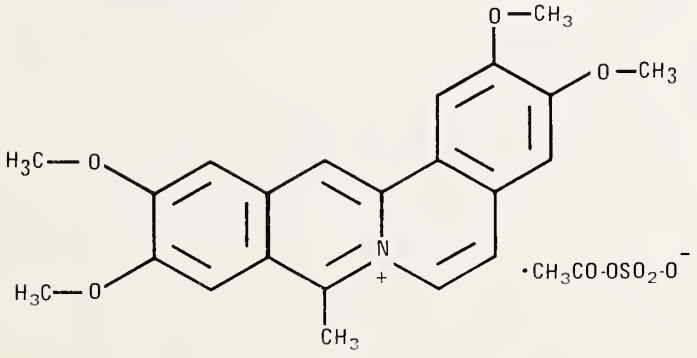


Chlorozotocin
NSC-178248

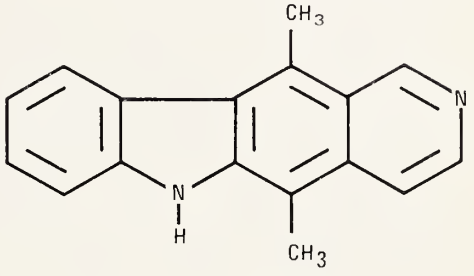
TEXT-FIGURE 4B.—Some unique compounds in development (continued).



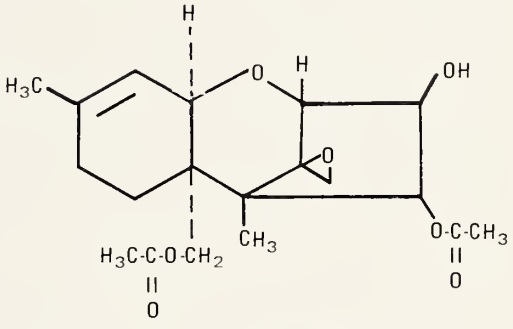
5 - Methyl - tetrahydrohomofolic acid
NSC-139490



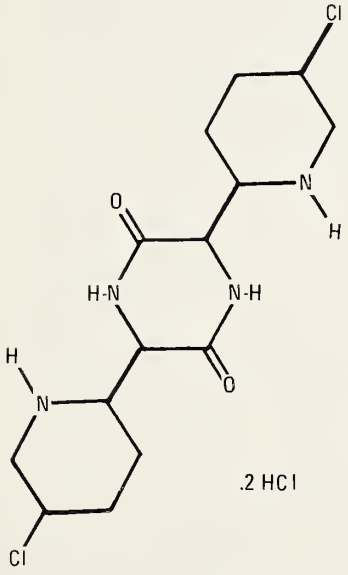
Coralyne sulfoacetate
NSC-154890



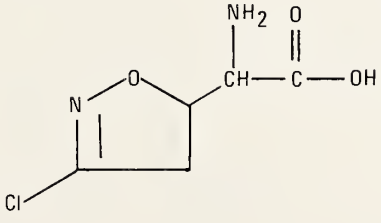
Ellipticine
NSC-71795



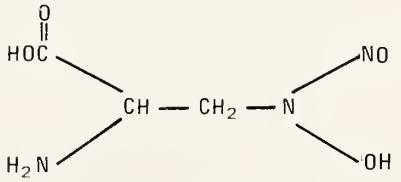
Anguidin
NSC-141537



Piperazinedione 593 A
NSC-135758

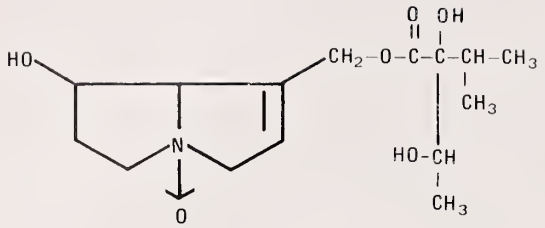


AT-125
NSC-163501

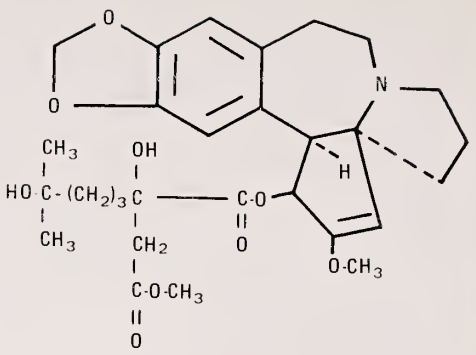


L-Alanosine
NSC-153353

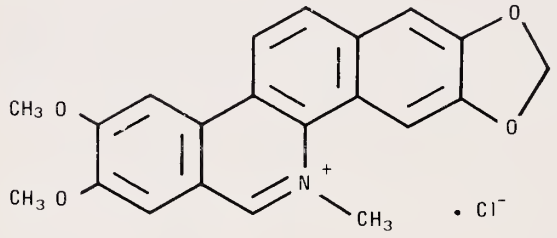
TEXT-FIGURE 4C.—Some unique compounds in development (continued).



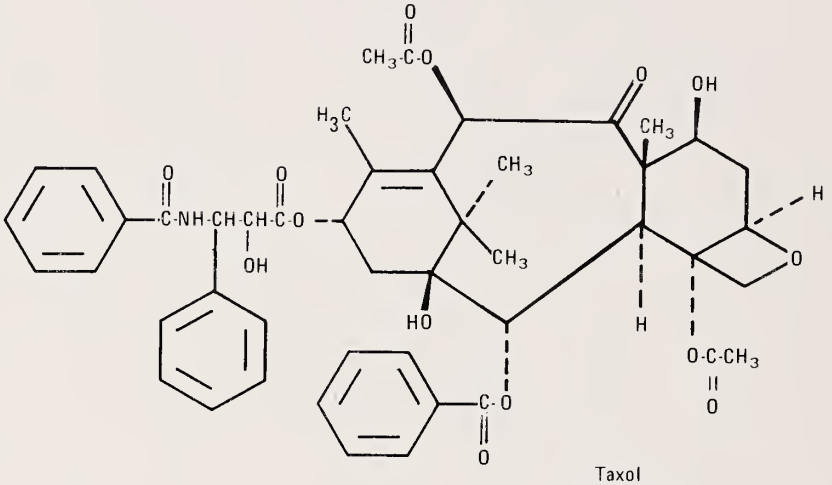
Indicine, N-oxide
NSC-132319



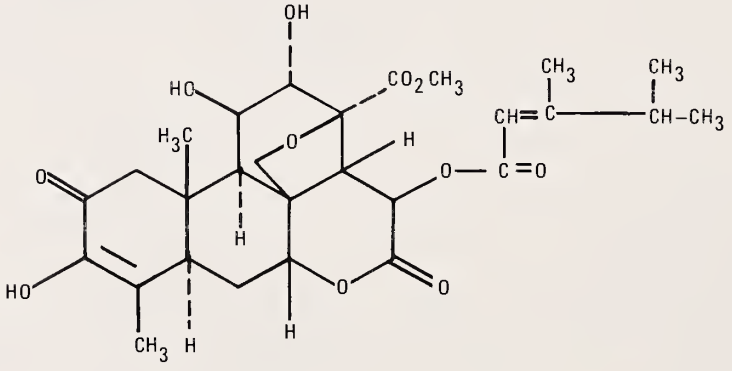
Homoharringtonine
NSC-141633



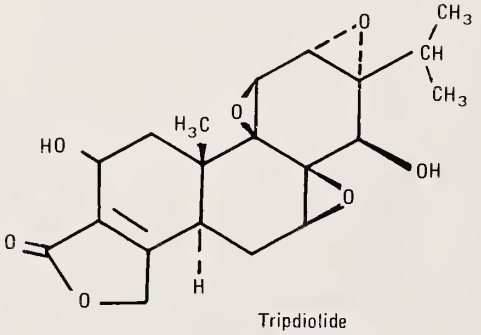
Nitidine chloride
NSC-146397



Taxol
NSC-125973

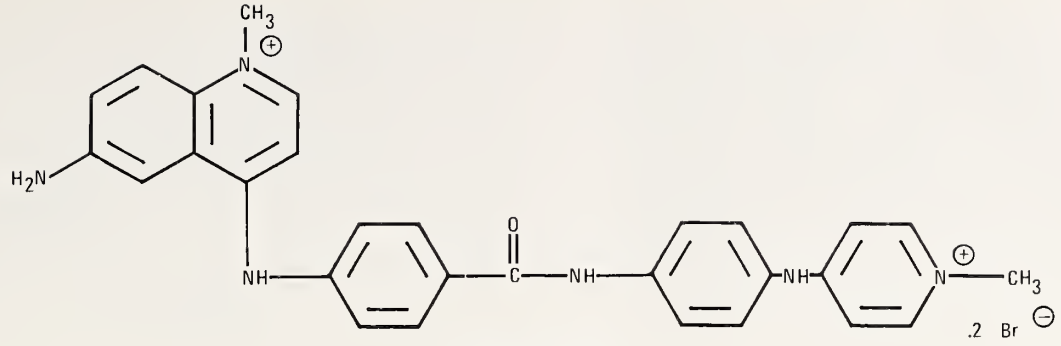


Bruceantin
NSC-165563

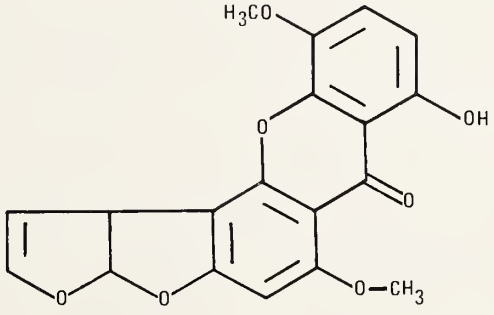


Tripdiolide
NSC-163063

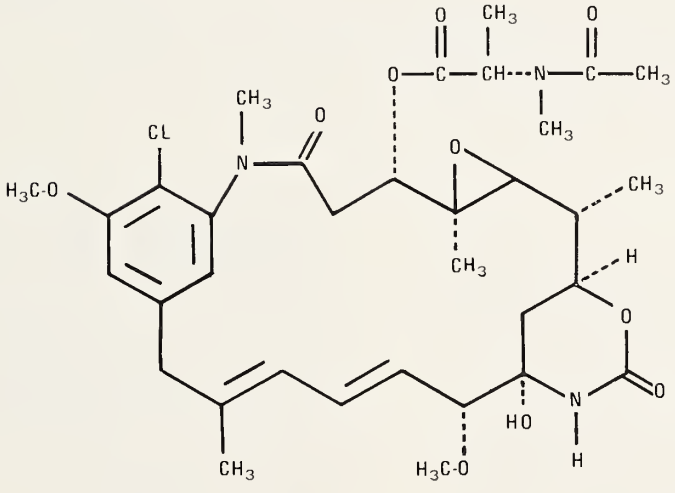
TEXT-FIGURE 4D.—Some unique compounds in development (continued).



Quinolinium derivative
NSC-176319

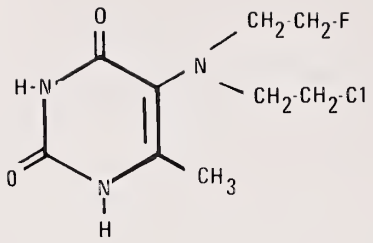


5–Methoxysterigmatocystin
NSC-178249

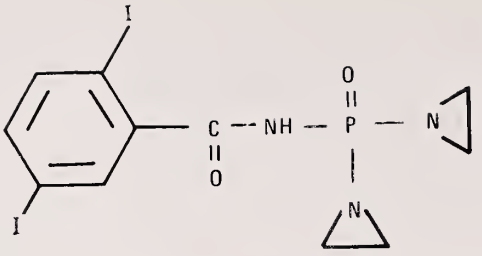


Maytansine
NSC-153858

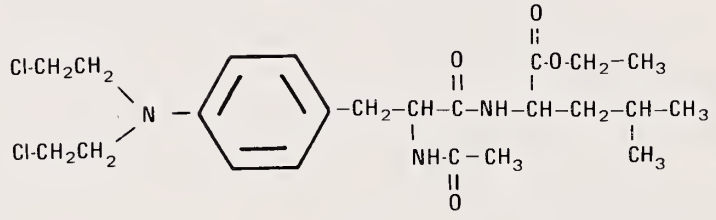
TEXT-FIGURE 4E.—Some unique compounds in development (continued).



Fluorodopan
NSC-73754



Diiodobenzotepa
NSC-167781



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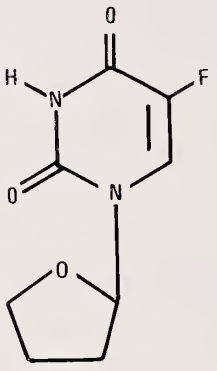

Ftorafur
NSC-148958

TEXT-FIGURE 4F.—Some unique compounds in development (continued).

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Screening at the National Cancer Institute

Abraham Goldin,[1] John M. Venditti,[2] *and* Stephen K. Carter[3]

The primary objective of screening programs in the field of cancer chemotherapy is to find agents that will exert clinical antitumor activity. To identify these potential agents, the screening systems must be sufficiently quantitative so that an initial observation of activity is readily reproducible. The initial screen should not be too sensitive, because this would result in the selection of too many positive agents, leading to an excess of "false positives." At the same time, the systems should not be too insensitive and fail to identify potentially useful compounds or "false negatives." In short, the screening and evaluation systems should have a maximum likelihood of classifying compounds as true positives and true negatives. They should provide the means for detailed agent characterization so that a decision can be made concerning its worthiness for further development, and, most important, there should be a strong probability that compounds active in the screening system will also be clinically active.

The successful accomplishment of these aims requires a methodology in which procedures are carefully detailed. These methods are illustrated by the original and subsequently revised "Protocols for Screening" (*1, 2*) of the National Cancer Institute (NCI). Included in these reports are methods for testing in transplantable tumors and cell cultures, as well as in other systems. The pertinent aspects will be summarized in this section.

Recently, the in vivo screening systems, including leukemia L1210 and leukemia P388, have been revised to accommodate the principles of a miniscreen, i.e., an econoscreen (cf pp 43, 147–150, 156) that will provide economies in materiel, time, and money. In addition, new systems are being investigated for their applicability to the identification of compounds that may exert therapeutic activity against various categories of human solid tumors, two of which [the Lewis lung (LL) carcinoma and B16 melanoma] have been useful in identifying new agents of potential treatment value in man.

GENERAL APPROACHES IN SCREENING

Animals

It is highly desirable that experimental animals be obtained from accredited breeders or be bred under stringent conditions in colonies affiliated with the screening program. Accredited producers of laboratory animals should, as a minimum, use the management principles and standards of the Institute of Laboratory Animal Resources (ILAR) for the breeding, care, and management of laboratory animals (*3–5*). That the requisite inbred, hybrid, or outbred animals conform with the specific tumors used is most important. As an adjunct to the program, there should be facilities for the antigenic typing of tumors and for characterization of the histocompatibility of the tumor and corresponding host.

The animals used in the tests may be of either sex. However, only one sex should be employed for any experimental test and should preferably come from a single source where the animals are of proven suitability.

The ILAR standards should govern animal maintenance and facilities. Conditioning rooms should be used to house animals when they are received. After the animals are removed from shipping crates and transferred to cages they must be fed, watered, and moved directly into quarantine rooms, which should be entirely separated from any source of contamination. Personnel assigned to quarantine rooms should not also work in the screening laboratories. When possible, the shipment of mice from each source should be maintained in a separate room. In fact, it is recommended that two or more shipments from one source, or several sources, not be pooled. Source identification records should be maintained, including date of receipt, age, sex, type of mouse, and other pertinent information. Placing additional animals in cages where previously there have been dead or moribund animals is not advisable.

The animals must be quarantined for at least 1 week after arrival. Then they are placed in sanitized cages and transferred to a holding room. Animals of usable weight and age that have been delivered by an accredited local supplier in an air-conditioned vehicle are transferred directly to clean cages supplied with fresh food and water and held overnight before use. If, in any instance, more than 10% of the animals die during the quarantine period, consideration must be given to rejecting that particular lot of mice.

Separate holding rooms should be provided for each species to maintain animals that have been in quarantine until they reach the required weight and age for experimental screening. If, after 3 weeks in the holding area, the animals have not reached an appropriate weight, they should be discarded. It is permissible to randomize the animals in the holding room just before their use in the screening tests.

Testing rooms are used only for the animals (only one species/room) that are on screening tests and should not be used to store such items as caging equipment, laboratory equipment, food, or bedding, etc.

Tumor donor rooms are used exclusively to maintain animals that serve as tumor donors. Each room should house only one animal species, but this does include two or more strains of the species. The food and bedding in each room should be kept in closed containers, and a sink and necessary equipment for routine animal care should be available. The utensils, food, bedding, etc., should not be transferred from one tumor donor room to another. Personnel in these rooms must exercise a high degree of care in sanitation and personal hygiene as a prerequisite for the appropriate care of the animals. A pan containing a foam rubber or rope pad saturated with a bactericide should be kept at the doorway of each animal room, and personnel must walk through it before entering the room. The tem-

[1] Chief, Laboratory of Experimental Chemotherapy, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014.

[2] Chief, Drug Evaluation Branch, Division of Cancer Treatment.

[3] Formerly, Deputy Director, Division of Cancer Treatment. *Present address:* Director, Northern California Cancer Program, Palo Alto, Calif. 94304.

perature in animal rooms should be maintained at between 73° and 75° F for mice and rats and between 70° and 75° F for hamsters; relative humidity should range from 45 to 55%. The receptacles, instruments, and utensils in the tumor donor rooms should be thoroughly sanitized and, when possible, sterilized before being used for a different group of animals.

Animal cages should be constructed of corrosion-resistant materials, and those used for quarantine and holding should have solid sides and bottoms. The maximum number of animals per cage and the recommended minimum floor space per animal for quarantine and holding are given in table 1 (*1, 3*). For drug testing, cages with solid sides and bottoms are also preferable but suspended cages with open mesh floors are acceptable. The recommended maximum number of animals per cage and minimum floor space per animal for drug testing are also given in table 1.

The cages should have food holders of corrosion-resistant material for pelleted rations. The food should not be placed on the floor of the cage (except for hamsters). Water is generally provided in a closed system consisting of a clear glass bottle and stopper with a stainless-steel or glass tube. The various parts should be sanitized before refilling.

Excreta and serum samples should be examined routinely to determine the presence or absence of *Salmonella* or infectious ectromelia. For special diagnoses, a facility for histopathologic examination should be available. The animals may be sprayed with an insecticide on arrival if they are suspected of having ectoparasites. Also, insecticides may be applied to interior building surfaces to prevent and control parasites. To control ectoparasites, toxic compounds may be applied to the area either by paint brushes or low-pressure spraying devices, but in the latter case, it is mandatory to remove animals before spraying the room. Control measures for the prevention of the escape or ingress of animals must be taken; control by means of bait stations is considered more effective than trapping. Dead animals should be removed, preferably in disposable plastic bags.

In some laboratories, antibiotics are used to improve or maintain the health of stock animals. If insecticides or antibiotics have been used, it is important to determine that these have not altered the response of animals to the drug employed in treatment or to tumor growth when they are placed on experimental test. During testing, application of insecticides or antibiotics is wholly undesirable.

Killing laboratory animals should be accomplished rapidly and in a humane manner; decapitation, anesthesia, air embolus, CO_2 asphyxiation, etc., are acceptable ways. It is important that the method selected not alter or damage the tumor or other tissues involved in the test.

Experimental Tumors

A wide range of tumor systems has been used in the primary and secondary screening programs (*3, 6, 7*) at NCI (tables 2, 3). A representative series of tumors maintained in the Drug Research and Development Program, the strain for propagation, the day of transfer, and the strain for testing are listed in table 4 (*1, 3*).

It is important to characterize each tumor histologically and to determine its growth pattern. Tumor samples should be frozen and stored in a tumor bank for renewal of the sublines and to provide a basis for comparison with the sublines in use. In the Drug Research and Development Program, tumor sublines are renewed from the tumor bank periodically. A record is maintained of the tumor transfer generations, both in the tumor bank maintenance center and in individual laboratories.

The techniques used in tumor transplantation are particularly important. It is necessary to transplant the tumor under aseptic conditions, preferably with the protection of a glass hood. After the donor animals are killed, they are bathed in a bactericidal solution, such as Zephiran, before removal of the tumor or ascitic fluid. Separate sterile instruments for removal of each solid tumor and individual sterile syringes for aspirating each of the ascites tumors are recommended. Ordinarily, for purposes of maintenance of carrier lines, the tumors are transplanted into the test site. Thus for the subcutaneous site, the tumors are ordinarily implanted in the axillary region; for the intramuscular site, they are usually injected into the thigh muscle. To obtain an ascites tumor, the transplant is usually injected ip. Both for the maintenance of the tumor line and for testing, the tumor material may be as trocar-implanted fragments or as cell suspension or cell brei, which (ordinarily) is injected by syringe. Caution must be exercised in the use of antibiotic treatment of animals during the maintenance of the

TABLE 1.—*Maximum number of animals per cage and minimum cage floor space per animal* [a]

Species	Animals per cage	Cage floor space per animal, inches [2]
Cages for quarantine and holding		
Mouse	25	8.0
Rat	10	30.0
Hamster	15	12.5
Cages for experimental testing		
Mouse	10	8.0
Rat	6	30.0
Hamster	6	12.5

[a] *See (1, 3).*

TABLE 2.—*Test systems that have been used as primary screens by Drug Research and Development* [a]

Classification	Tumor system
Mouse tumors	Adenocarcinoma 755
	Cloudman melanoma (S91)
	Ehrlich ascites
	Friend virus leukemia
	Hepatoma 129
	Lewis lung carcinoma
	Lymphoid leukemia L1210
	Osteogenic sarcoma HE10734
	Sarcoma 180
Rat tumors	Dunning leukemia ascites
	Dunning leukemia solid [b]
	Human sarcoma HS1
	Murphy-Sturm lymphosarcoma
	Walker 256 (intramuscular)
	Walker 256 (subcutaneous)
Hamster tumors	Adenocarcinoma of duodenum
	Adenocarcinoma of endometrium
	Adenocarcinoma of small bowel
	Melanotic melanoma
Human tumor	Human sarcoma HS1

[a] *See (3,6).*
[b] For alkylating agents only.

TABLE 3.—*Test systems that have been used as secondary screens by Drug Research and Development* [a]

Classification	Tumor system
Mouse	Adenocarcinoma E0771
	Adenocarcinoma 755 (ascites)
	ADJ-PC-20 plasma cell tumor
	Bashford carcinoma 63
	Ehrlich carcinoma
	Glioma 26
	Harding-Passey melanoma
	Hepatoma 128
	Leukemia B82
	Leukemia B82T
	Leukemia B82/FU (5-fluorouracil)
	Leukemia B82/38280 (terephthalanilide)
	Leukemia P288
	Leukemia P288/740 (amethopterin)
	Leukemia P335
	Leukemia P388
	Leukemia P388/38280
	Leukemia P388/49842 (vinblastine)
	Leukemia P388/67574 (vincristine)
	Leukemia P815
	Leukemia P815/49842
	Leukemia P815/FU
	Leukemia P815/FUR (5-fluorouridine)
	Leukemia P815/38280
	Leukemia P1081 chloroleukemia
	Leukemia 1210/P3771
	Leukemia 1210 intracerebral inoculation
	Leukemia 1210/M-GAG
	Leukemia 1210/38280
	Leukemia 1210/AZA (8-azaguanine)
	Leukemia 1210/TG (6-thioguanine)
	Leukemia 1210/MP (6-mercaptopurine)
	Leukemia 1210/MP/A (6-mercaptopurine and amethopterin)
	Leukemia 1210/azaserine
	Leukemia 1210/FU
	Leukemia 1210/AMPFU$_2$
	Leukemia 1210/cytosine arabinoside
	Leukemia L4946 (solid)
	Leukemia L4946/742 (azaserine)
	Leukemia B8174T
	Lewis bladder carcinoma
	Lymphoid leukemia 4K$_4$
	Lymphoid leukemia P1534
	Lymphoid leukemia 5178Y
	Lymphoma 2
	Lymphoma 4
	Lymphosarcoma 6C3HED (Gardner)
	Lymphosarcoma mecca
	Lymphosarcoma P1798 (survival)
	Lymphosarcoma P1798/cortisone
	Miyono adenocarcinoma
	MPC-2 plasma cell tumor
	Myeloid leukemia C1498
	Plasma cell LPC-I
	Reticulum cell sarcoma
	Ridgway osteogenic sarcoma
	Sarcoma 37
	Sarcoma 180 (ascites)
	Sarcoma 180/A
	Sarcoma 180/6-MP
	Sarcoma 180/AZA
	Sarcoma 180/6-MP-AZA
Mouse—continued	Sarcoma T241
	Wagner osteogenic sarcoma
Rat	Babcock kidney tumor
	Dunning leukemia ascites/Cytoxan
	Dunning leukemia ascites/29189 (thiopurine)
	Dunning leukemia solid/HN$_2$
	Dunning leukemia ascites/thioguanosine
	Flexner-Jobling carcinoma
	Iglesias functional ovarian tumor
	Iglesias sarcoma (originally ovarian)
	Jensen sarcoma
	Mason mammary adenocarcinoma
	Novikoff hepatoma
	Walker 256 (subcutaneous)
	Walker 256 (pulmonary)
	Walker 256/sc/Cytoxan
	Yoshida reticulum cell sarcoma
	Lymphoma 8
Hamster	Adenocarcinoma of the breast
	Adenocarcinoma of the duodenum
	Adenocarcinoma of the kidney
	Adenocarcinoma of the pancreas
	Adenocarcinoma of the prostate
	Amelanotic melanoma
	Carcinoma of the adrenal cortex
	Carcinoma of the pituitary
	Crabb hamster sarcoma
	Cystadenocarcinoma of the liver
	Fibrosarcoma No. 1
	Leiomyosarcoma
	Liposarcoma
	Malignant neurilemmoma
	Osteogenic sarcoma
	Plasmacytoma No. 1
	Plasmacytoma No. 2B
	Plasmacytoma No. 1/409962 [urea-1, 3-bis-(2-chloroethyl-1-nitroso-)]
	Plasmacytoma No. 1/9706 (triethylenemelamine)
	Reticulum cell lymphosarcoma
Chicken	Rous sarcoma
Induced	3-Methylcholanthrene-induced mammary adenocarcinoma
	Dimethylbenzanthracene-induced mammary adenocarcinoma
	3,4,9,10-Dibenzypyrene-induced fibrosarcoma
Tumors in heterologous hosts	Human amelanotic melanoma in conditioned hamsters
	Human myxofibrosarcoma in conditioned hamsters
	DBA/2 mouse lymphatic leukemia in conditioned hamsters
	HS-1 in conditioned rats
	HEP-3 in conditioned rats
In vitro systems	Hamster adenocarcinoma of the duodenum
	HEP-2 (human epidermoid carcinoma)
	HEP-2/755 (6-mercaptopurine)
	HEP-2/740 (methotrexate)
	Lysogenic induction
	Rous sarcoma

[a] *See (3, 6, 7).*

tumor lines, because this treatment may alter the tumor or its response to therapeutic agents. In any individual chemotherapeutic test, it is desirable to transplant tumor material taken from the same transfer generation.

When tumor fragments are used, their size should be standardized. For various tumors, the usual fragment ranges from 2 to 6 mm. A sterile petri dish, containing buffered physiologic saline or an equivalent medium, is generally used for holding tumor fragments, which are kept cold in an ice bath.

Generally, a sterile 13-gauge trocar is used for implanting the fragment under aseptic conditions. One tumor is often sufficient for implantation of approximately 30 mice, 60 ham-

TABLE 4.—*Tumors, host strains, and transfer times for a series that have been maintained by Drug Research and Development*[a]

Tumor	Strain for propagation	Transfer day	Strain for testing
Mouse			
Sarcoma 180	Outbred albino	6-7	Outbred albino
Adenocarcinoma 755	C56BL/6 female	12-14	BDF_1
Lymphoid leukemia L1210	DBA/2	6-7	"
Leukemia L1210/methotrexate	"	6-7	"
Lewis lung carcinoma	C57BL/6	12-14	"
Cloudman melanoma (S91)	DBA/2	21	"
Friend virus leukemia	"	13-14	"
Osteogenic sarcoma HE10734	C3Hf	18-21	C3H/He
Hepatoma 129	C3H/He	14	C3H/He (or hybrid)
Rat			
Walker 256 (sc)	Suitable outbred	11-13	Suitable outbred
Walker 256 (pulmonary)	"	11-13	"
Walker 256 (im)	"	10-12	"
Dunning leukemia ascites	F344	7	F344
Dunning leukemia solid	"	10	Suitable outbred
Murphy-Sturm lymphosarcoma	Suitable outbred	12-14	"
Human sarcoma HS1	"	12-14	"
HEP-3	"	10	"
Hamster			
Adenocarcinoma of small bowel	Hamster	10-12	Hamster
Adenocarcinoma of endometrium	"	10-12	"
Melanotic melanoma	"	16	"
Plasmacytoma	"	16	"
Plasmacytoma/Cytoxan	"	16	"
Adenocarcinoma of duodenum	"	5-6	"

[a] *See (1,3).*

sters, or 90 rats. Tumor preparation and implantation should be accomplished within a 30-minute period or in as short a time as possible; fragments from each tumor are tested for bacterial contamination. Usually, separate holding cages are used for each set of animals implanted with material from a single tumor. When it is determined that the 24-hour observation of bacterial cultures is negative, animals may be randomized to the test groups.

In tumor brei preparation, the tumor is excised under aseptic conditions, minced with sterile scissors or a tissue grinder or expressed through a sterile tissue press into a sterile glass container, and sterile saline is added in the desired amount. Like the tumor fragments, the tumor brei is kept in a sterile glass container in an ice bath.

It is important to accomplish the initial removal of the tumor, preparation of brei, and implantation into recipient animals within 30 minutes. Usually, each animal receives an inoculation of 0.2 ml of the tumor brei; syringes which should not be refilled are equipped with no larger than 19-gauge needles. One brei can be used for implanting approximately 60 animals. Samples of the brei are cultured in microbiologic media to ensure that it is not contaminated. Separate holding cages are used for each group of animals implanted from one brei preparation. The animals are not randomized into experimental groups until the results of the 24-hour bacterial cultures prove that the brei is not contaminated.

Ascites tumor preparations are generally prepared for leukemia cells. The fluid is withdrawn aseptically by insertion of a needle through the abdominal muscle from which the skin has been removed. After collection, the ascitic fluid is kept in a sterile glass container in an ice bath; samples are pooled if more than one donor animal is used. An aliquot of ascitic fluid is placed in a separate container with heparin for cell morphology and determination of the cell count. In the morphologic examination, a drop of fluid is expressed from the syringe on a glass slide for a smear, which is stained with Wright's for a differential leukocyte count; the fluid must contain more than or as much as 95% tumor cells. The remainder of the fluid in the syringe is placed in a small test tube containing dry heparin and diluted 10- to 100-fold with saline. The same procedure is used for cell counts as for white blood cell determination.

During implantation, the sterile ascitic fluid (kept in an ice bath) is usually diluted with sterile physiologic saline plus glucose, so that the desired inoculum is contained in 0.1 ml. Other suitable diluents include Hanks', Locke's, Gey's, Earle's, or Tyrode's. The diluted fluid is then injected ip with a 23-gauge needle, and new sterile syringes and needles are used for each refill from the pool of donor fluid. Not more than 60 minutes should elapse from the time the fluid is taken from donors until all test animals receive the injections.

The first four drops of undiluted fluid from the syringe containing the ascites cells of each donor animal is tested for bacterial contamination. The cell counts and morphology studies are conducted with individual or pooled fluid. Bacterial cultures are read at 24 hours, and if they are contaminated, the test is discarded.

Quality Control

Quality control procedures are necessary to ascertain that tumors do not harbor bacterial contaminants; tests are conducted for each tumor used to maintain the stock tumor line as well as those for the preparation of inocula for drug testing. The tests are usually conducted in quadruplicate, either with fragments of solid tumors or with individual drops of undiluted ascites tumor; four tubes of thioglycolate broth are used per tumor. The tubes are incubated for 48 hours, half of them

at room temperature and the others at 37° C, and the cultures are read at 24 and 48 hours. When no contamination is evident, and also if growth has occurred in only one tube at the 24-hour reading at either temperature, the experimental test is performed. If there is bacterial growth in two or more tubes after 24 hours, the animals that have been implanted with tumor fragments or fluid should be discarded. Other animals prepared for testing for which the tumor material did not show contamination may be used for tests after rescheduling. When the inoculum consists of pooled ascitic fluid and there is any evidence of contamination, the whole group of animals is discarded. If, at the 48-hour observation period, bacterial growth has occurred in two or more of the cultures from one tumor, the test is also discontinued, and an effort is made to identify the contaminating organism.

The tumors used for drug evaluation should grow uniformly, show host compatibility, and be uniformly and reproducibly responsive to active chemotherapeutic agents. For solid tumors, the average tumor size of control groups should fall between required maximum and minimum limits on the day of evaluation. When solid tumors are used in survival time studies, the longevity pattern should be uniform and the average or median survival must likewise be within prescribed limits. For ascites tumors, the survival time of control animals given inoculations of a specified number of tumor cells should also fall within prescribed limits. If possible, it is advisable in test runs to inject a control set of animals with the assay inoculum and a series of dilutions of the inoculum to determine its potency.

There should be a high percentage of tumor "takes" in control groups, because an excessive incidence of "no-takes" indicates a serious problem pertaining to technique, the tumor, or the host. A no-take is defined as failure to observe progressive tumor growth in an animal after implantation of a solid tumor or inoculation of tumor cell suspension. A listing of excessive no-takes in controls for tumor systems in which tumor weight is the parameter of evaluation is given in table 5 (*1, 3*). When tumor growth occurs initially but is followed by regression in control animals, a problem may exist in the histocompatibility relationships of the tumor and host.

A number of factors may indicate that an experimental test is suspect; e.g., excessive weight loss in the animals or evidence of infection may indicate an invalid test. The test is also questionable if early deaths occur that are not attributable to progressive tumor growth. When untreated non-tumor-bearing animals are used as controls, weight loss or death indicates the existence of a serious problem. Any instance of a complication in the testing demands an investigation into the nature of the problem, taking into account the host and the tumor, or the experimental techniques involved in the various aspects.

TABLE 5.—*Excessive no-takes in controls for tumor systems in which evaluations are based on tumor weight* [a]

Animals	Number of surviving animals	Tumor weight considered as no-takes, mg	Considered as excessive no-takes
Mice	15–24	≤39	≥3
Rats	25–34	≤99	≥4
Hamsters	35–44	≤39	≥5
	45–54		≥6

[a] *See (1, 3).*

As an adjunct to untreated controls, a positive control standard offers a considerable advantage in the conduct of screening tests. The positive control ordinarily is a well-known compound with high and consistent activity in the particular tumor system used. When diminished or excessive activity is observed for the positive standard, even though the untreated control animals may have behaved in a uniform manner, the testing could be suspect.

In special test systems, tumors specifically resistant to a compound may be used for studies of cross-resistance, and in addition to untreated controls and a positive standard, another control should be included in which animals are treated with a compound to which the tumor is resistant (negative standard).

Drug Identity, Purity, and Dosage Form

Identity and purity tests involving melting point determinations and elemental, chromatographic, and spectrophotometric analyses are conducted for new drugs before their use. The dosage form, developed for preclinical and clinical use, requires a determination of solubility at room temperature in water, saline, 5% dextrose, or appropriate physiologically acceptable solvents. If the drug is insoluble at room temperature, an attempt may be made to dissolve it with moderate heat. The dosage form is characterized with respect to the pH of solution, isotonicity, and retention of solubility on standing. The stability of the bulk drug and dosage form is checked routinely, and any deleterious effect of sterilization is determined. As a part of the toxicology protocol, the compatibility of the formulation with human serum and plasma is characterized.

Preparation and Administration of Test Materials

In the usage of synthetic compounds, physiologic saline or distilled water should be used for soluble compounds. Another suitable solvent or suspending agent is required for compounds insoluble in water (or saline). Possible diluents include alcohol or acetone, diluted with physiologic saline, distilled water, 0.5% carboxymethyl cellulose (CMC), or hydroxypropyl cellulose (Klucel). A final concentration of alcohol exceeding 2% or acetone exceeding 5% is considered deleterious. If the compound is soluble in acid, hydrochloric acid (0.1 N) may be added. Alkali-soluble compounds may be dissolved in sodium bicarbonate, sodium carbonate, or sodium hydroxide. If acid or alkali is used as the diluent, it is advisable to titrate back to a pH of 4.5 to 9, when possible.

When the compound is insoluble, CMC, Klucel, or another suitable suspending agent may be added, but it should be of such nature that a relatively clear or uniform suspension is obtained. The suspending agent should have a viscosity rating of 2,000 centipoise or greater. It is possible to obtain a uniform suspension by careful grinding with a mortar and pestle, homogenizers, or sonifiers, and Tween 80 (1 or 2 drops) may be added if needed to stabilize an emulsion. Care should be exercised in the use of heat (≥60° C) or organic solvents, as well as surfactants like Tween 80, since these procedures may lead to destruction or alteration of the test compound. Olive oil, peanut oil, or other types of vehicles may be used when necessary. It should be remembered that unstable compounds must be prepared immediately before each injection period.

Antibiotic filtrates, which can be diluted with physiologic saline, distilled water, or an appropriate medium must be stored at temperatures of −15° C or below. Before use, fil-

trates are thawed at room temperature until they are slushy and then kept at refrigerator temperature. They are warmed to room temperature (not exceeding 25° C) just before injection, but not for extended periods. Although care must be exercised, any remaining filtrates may be used again if they are refrozen rapidly.

Plant materials and other natural products received in the form of alcohol extracts concentrated to a semisolid consistency may be ground with the addition of small quantities of sterile saline or 95% ethanol before dilution in an appropriate vehicle such as CMC. When the plant materials or other natural products have been frozen, they may be treated in a manner similar to antibiotic filtrates. If the aqueous extracts have been lyophilized, they may be dissolved in saline or suspended in 0.5% CMC or an appropriate suspending agent.

Although numerous routes may be selected for the administration of test materials, synthetic compounds, antibiotic filtrates, and other natural products are generally injected ip or sc. Additional routes include oral, im, iv, and intracranial (ic) administration. The quantity of diluent in which the dose is contained should not exceed that which can be tolerated by the host. Recommended volumes (drug plus diluent) for injection in mice, rats, and hamsters are shown in table 6 (*1, 3*). If the recommended volume must be exceeded, the dose may be divided and administered at several sites or times to permit appropriate absorption. When the test material is suspended in oil, limiting the volume to approximately 0.2 ml for all species is recommended. In any event, it is advisable to treat control animals with an equal volume of the vehicle used for administration of the test compounds.

TABLE 6.—*Recommended volumes in milliliters (drug plus diluent) for injection* [a]

Type of injection	Mice	Rats	Hamsters
Intraperitoneal			
Fermentation products	0.5	1.0	1.0
Other materials	0.2	1.0	0.5
Oral	0.2	1.0	1.0
Subcutaneous	0.2	1.0	1.0

[a] *See (1, 3).*

Drug Selection and Common Schedules

Before dose selection for a new compound, preliminary toxicity studies are frequently performed and may be followed by dose–response testing conducted in a single experiment or sequentially. Alternatively, it is possible to attempt to cover an appropriate dose range extending from nontoxic to toxic doses in either dose–response or sequential testing. It was the general practice in the sequential system NCI used for a time to inject a daily dose of 500 mg/kg in the initial test when toxicity data were not available. Currently, when toxicity data are not supplied and a sufficient quantity of the compound (≥220 mg) is available, testing is initiated with a dosage of 400 mg/kg as the highest dose. When dosage cannot be expressed in dry weight, as with a material supplied as a dilution, the total volume of test injection is usually 1 mg/animal. In this instance, the material is administered at the concentration provided and also at a series of dilutions.

In a tumor inhibition study, a drug dosage is considered toxic if 34% of the animals die by the day they are to be killed. If survival time is the parameter of response, a test is considered toxic if 1) 34% of the animals are dead at a designated time after initiation of treatment (i.e., day 5 for leukemia L1210 when treatment is initiated early), 2) the ratio of the survival time in treated animals (T) compared with untreated controls (C) or T/C is less than or equal to 85%, or 3) there is excessive animal weight loss relative to controls at a designated time after therapy is initiated. For leukemias L1210 and P388, when drug treatment is initiated 1 day after leukemia cell inoculation, excessive weight loss has occurred if the weight of the treated animals is more than or equal to 4 g less than that of controls. When acute toxicity occurs, the subsequent dosage may be reduced by 50% or more in accordance with the extent of observed deaths and is generally reduced by half after the occurrence of chronic toxicity.

Common schedules of administration may include single daily treatment, a number of daily treatments, or intermittent treatment. Drug administration by continuous infusion is also employed in special cases. It is possible to vary the number of treatments, their spacing, and the total duration of treatment. All these factors may influence drug effectiveness.

Size of Test and Control Groups

The number of animals included in the control and test groups depends on the reproducibility of the tumor system and the nature of the experimental design. The desirable number of animals per group may vary according to the number of dose levels for each compound and the number of compounds tested at one time. The recommended control and test group size used by NCI in a protocol specifying 6 or 10 animals per test group is shown in table 7 (*1, 3*). Appropriate adjustments may be made for a reduction in the number of animals (*1*).

TABLE 7.—*Control and test group size* [a]

Schedule	Number of actual test groups (or dose levels)	Number in each treatment group	Number in controls
A[b]	1-6	8	18
	7-21	7	30
	22-25	6	30
	26-30	6	33
B[c]	1-4	16	24
	5-13	12	33
	14-24	10	40
	25	10	43

[a] *See (1, 3).*
[b] Schedule used when protocol specifies 6 animals/test group.
[c] Schedule used when protocol specifies 10 animals/test group.

Randomization Procedures

Drug evaluation requires randomization of animals in accordance with standard procedures in several systems. By one method, sheets of random numbers are prepared for the scheduled treatment groups. The animals are picked at random from a holding cage and assigned to treatment groups in the order in which the numbers are listed. In the index card method, cards are prepared with each representing an animal in a control or experimental treatment group. Numbers are assigned from a table of random numbers and the mice are distributed accordingly. If precise comparisons are to be made, it may be important to randomize not only the animals but also the order of

injection. Other types of procedures may be used that stratify animals by weight, sex, or other designated characteristics before randomization.

Screening and Evaluation Systems

The lymphoid leukemia L1210 system is currently the standard screen in the NCI program for initial testing of synthetic compounds, whereas the lymphocytic leukemia P388 is similarly used for natural products. Melanotic melanoma B16 and LL carcinoma, under investigation as systems applicable to the selection of compounds that may be active against solid tumors in man, are used in tests of compounds found active in leukemia L1210 and/or P388 and in special assays. The Walker carcinosarcoma 256 was once employed in routine screening and now is used in special testing and, in certain instances, in detailed investigations of compounds of interest. The protocols for lymphoid leukemia L1210 and lymphocytic leukemia P388 (before their modification in the miniscreen or econoscreen to be discussed later), melanotic melanoma B16, LL carcinoma, and Walker carcinosarcoma 256 are summarized in appendix III (*2*).

The in vitro KB cell culture system, which is described in appendix III, is also used routinely to determine whether a test agent has cytotoxic action or is capable of inhibiting cell growth.

The in vivo test systems have either tumor weight inhibition or survival time of animals as the criteria of effectiveness. Ordinarily, for studies that involve tumor weight, the mean tumor weight is determined and the ratio (T/C) is computed. T/C is the quotient (expressed in percent) of the mean tumor weight for control animals. The survival time of the animals, generally calculated as the mean or the median survival time, is usually expressed as T/C, i.e., the survival time of treated animals divided by the survival time of control animals.

The systems for screening are constantly being revised and improved in accordance with progressive experience and the needs of the program. In addition, investigations are continually in progress to discover new model systems and ascertain their application in the detection of clinically active compounds.

Combination Chemotherapy and Other Special Studies

There is strong clinical interest in the treatment of human neoplasia with combinations of drugs. At NCI attention is focused on attaining a more extensive chemotherapeutic response or "therapeutic synergism" by drug combinations as compared with individual compounds. In experimental studies, measurement of response may be through tumor inhibition, survival time, or percentage of survivors, and it is important to compare the dose–response curves for the drug combination with those for the individual compounds and to determine whether therapeutic synergism has been achieved by the combination. A description of the detailed methodology in this area is available in several review articles (*8, 9*).

Detailed methodology has also been developed for studying the influence of drugs in intracranial disease, the origin of drug resistance, cross-resistance between agents, collateral sensitivity, immunotherapy plus chemotherapy, and surgery plus chemotherapy, as well as for other modalities of treatment.

MINI-ECONO-SCREENING SYSTEM

Recently, an important need has arisen for definitive methodology for in vivo testing of small quantities of chemical agents in the NCI screening program. This requirement is largely due to refinements in the techniques for identifying chemical structure, which can now be accomplished with small amounts of compound. As a result, the number of new compounds of potential interest to the program is increasing, but chemists are understandably reluctant to synthesize these in larger quantities after their chemical structure and purity have been determined.

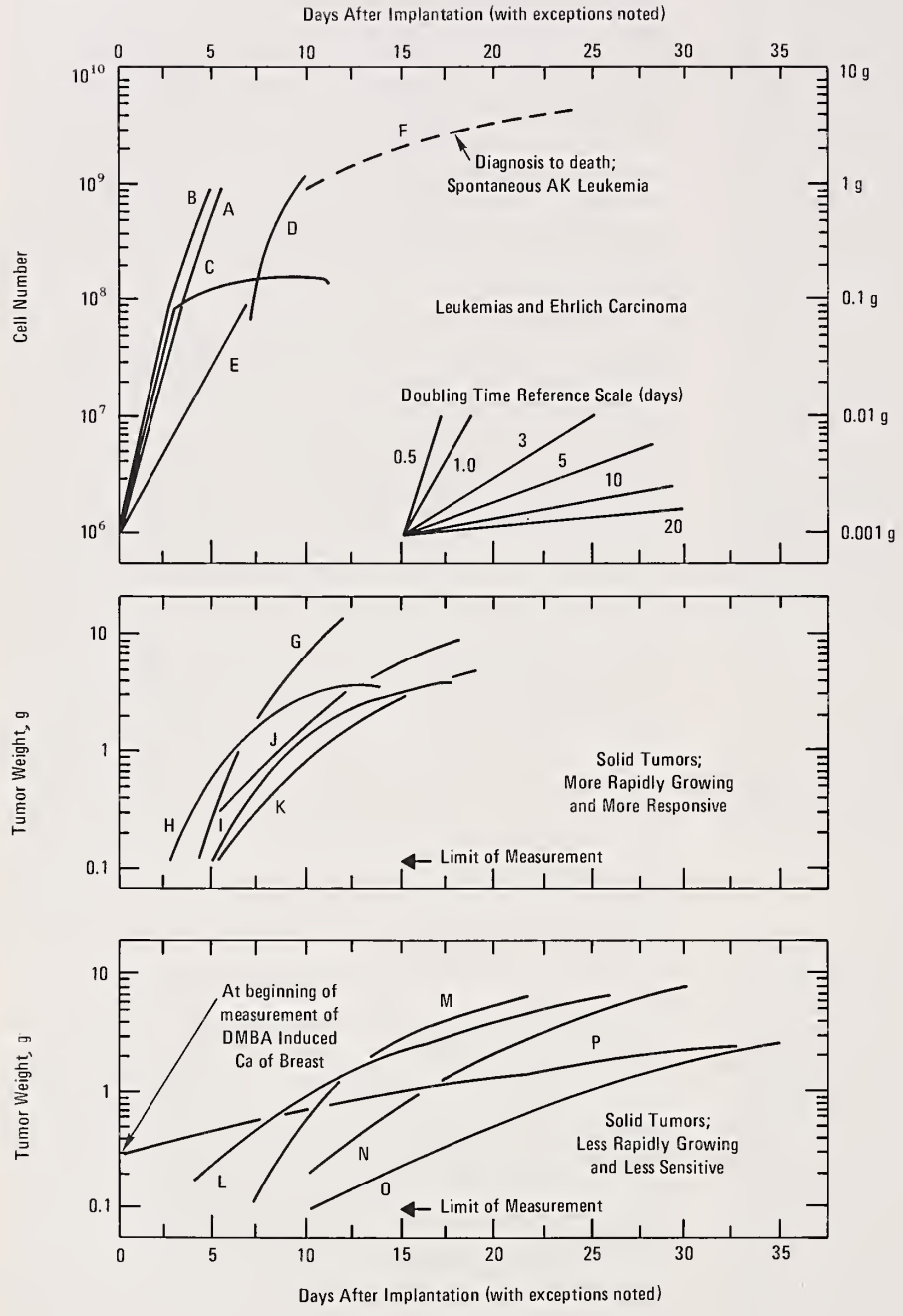
Thus a screening approach for agents in small amounts affords an opportunity to test small amounts of materials that otherwise could not be assayed in vivo; among these, particularly the recently synthesized compounds, could be chemical structures of the most interesting and novel types. Furthermore, the use of minimum quantities in initial screening permits retention of sufficient amounts for additional tests, both in the initial system and in others in the program. Finally, the system is highly economical in the expenditures of time, labor, and material, which permits a marked cost reduction in this facet of the program.

The concept of the mini-econo screen was developed (*10*) to meet these needs in the NCI program and has been affixed to the L1210 and P388 systems for synthetic materials and crude natural products, respectively. The modified protocols, encompassing the mini-econo-screening concept for leukemia L1210 testing (*11*) are presented in appendix III.

In the most recent developments as outlined by Dr. S. A. Schepartz (unpublished observations) in appendix III, the Division of Cancer Treatment (DCT) intends to place greater emphasis on the testing of new synthetic compounds and natural product isolates against a spectrum of animal models of specific human tumors. The striking improvement in the therapy of human cancer in recent years can be attributed to a number of factors including the discovery of more effective anticancer agents, better use of older drugs, discovery of effective drug combinations, advances in techniques of patient supportive care (*12, 13*), and to the increasingly productive interaction of clinical scientists with laboratory scientists (*14, 15*). It has also been observed that the most rapidly growing animal and human tumors are considerably more sensitive to chemotherapy than are slow-growing tumors. Thus progress in human anticancer chemotherapy has been primarily against the relatively rapid-growing leukemias and lymphomas that do not grow as large discrete masses and are characterized by a relatively high proliferative fraction (PF), which is the percentage of viable tumor cells synthesizing DNA and undergoing replication at any given time.

Research projects conducted within the DCT Drug Development Program (by Drs. H. E. Skipper, F. M. Schabel, Jr., et al., Southern Research Institute) have revealed that ascites L1210 exhibits a short population doubling time (TD) of about 0.5 day and a high PF of over 80%. L1210 is exquisitely sensitive to specific inhibitors of DNA synthesis, i.e., the S-phase specific drugs. The corollary that drugs that inhibit other specific phases of the cell cycle or all phases of the cell cycle are not active against L1210 does not, however, appear to be true. Nevertheless, the specific S-phase inhibitors (antimetabolites) would not be expected to be active against a solid tumor with a relatively low PF; in general, they are not.

The program has made an intensive effort to characterize a number of potential animal tumors with respect to their growth characteristics with the expectation that such models might aid in selecting, from among the drugs uncovered in the L1210 screen, those with the greatest potential for efficacy against the slow-growing human solid tumors (*16–22*). Text-figure 1 compares the growth rates of 16 animal tumors (*23*). Each



TEXT-FIGURE 1.—Growth rate comparisons of various experimental animal tumors (*23*). A= L1210 leukemia (ip), mouse; B= L1210 leukemia or long-passage AK leukemia (iv), mouse; C= Ehrlich ascites carcinoma (ip), mouse; D=solid L1210 leukemia (sc), mouse; E=first-passage AK leukemia (iv), mouse; F=advanced spontaneous AK leukemia (estimated), mouse; G=Walker carcinoma 256 (sc), rat; H=sarcoma 180 (sc), mouse; I=carcinoma 755 (sc), mouse; J=plasmacytoma (sc), hamster; K=solid Ehrlich carcinoma (sc), mouse; L=Lewis lung (sc), mouse; M=B16 melanoma (sc), mouse; N=melanotic melanoma (sc), mouse; P=DMBA-induced carcinoma of breast, rat.

falls into one of three groups: 1) the rapidly growing leukemias and lymphomas, exemplified by L1210; 2) the most rapidly growing of the solid tumors, exemplified by Walker 256, and 3) the slowest growing solid tumors. In the latter group, the slowest growing tumors are the first generation transplant of the C3H mammary carcinoma and the carcinogen-induced breast carcinoma. However, the operational characteristics of these tumors are such that relatively few drugs could be tested against them in the course of a year.

Among the slower growing tumors of interest as ancillary screens, as indicated above, are the B16 melanoma and LL carcinoma of mice. The cell-cycle parameters of L1210, B16, and LL, as well as the spontaneous AK leukemia and the carcinoma induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) are shown in table 8. Whereas the PF and TD of B16 or LL do not approach the low values characteristic of large human solid tumors (*22*), they are considerably lower than those for L1210. Thus B16 and LL are considered prime candidates for further study as potential screening tools.

In the course of studying the operational characteristics and responses to therapy of B16 (*17, 18*), it was apparent that the tumor implantation site markedly influences its sensitivity to drugs (table 9). Not only did inoculated B16 or sc-inoculated B16 exhibit its own distinct spectrum of responsiveness, but each responded to at least one important drug that was ineffective against the other. Adriamycin, daunorubicin, vinblastine, vincristine, and dactinomycin, all drugs with only moderate activity against L1210, were among the most effective against ip B16 and were relatively ineffective against sc B16. The antimetabolites [methotrexate, 5-fluorouracil (5-FU), 5-FUDR, and 6-MP] showed little or minimal effect against ip B16 but only when given on an intensive intermittent treatment schedule. However, the activity against ip B16 was minimal compared with its activity against L1210. Subcutaneous B16 responded to relatively few drugs, but among them was bleomycin, which was ineffective against ip B16 (tables 9, 10).

Interest in LL was intensified when it was observed that the tumor could clearly discriminate methyl-CCNU from BCNU and CCNU (*17*). All three of these nitrosoureas are curative against L1210. The superiority of methyl-CCNU against LL prompted its selection for development to clinical trial. LL, though clearly resistant to most drugs, was sensitive to bleomycin (Southern Research Institute: Unpublished data).

TREATMENT SCHEDULE DEPENDENCY OF EXPERIMENTALLY ACTIVE DRUGS

Once a compound has been found to be active in the screening system it becomes the subject of route- and schedule-dependency studies.

The principal objective of schedule-dependency tests in rodent tumor models is to obtain information on optimal conditions of drug use for designing toxicologic studies in larger animals and, ultimately, for use by clinicians. Secondly, schedule-dependency studies may furnish leads for uncovering biochemical sites of action, which, in turn, could lead to the development of curative combination chemotherapy. The tests also provide a broad data base for selecting a manageable number of treatment schedules for use in large-scale screening.

The increasing awareness of factors affecting antitumor specificity, particularly the relationship between drug effects and the phases of cell cycle, suggests that animal studies relating schedule dependency to the toxicologic, pharmacologic, and biochemical actions of the drug and to the growth and kinetics of malignant and normal cells can establish important principles for improving clinical treatment. Skipper, Schabel, and their colleagues (*25–27*) have emphasized the importance of the kinetics of tumor-cell proliferation, sites of drug action at the molecular level, and the maximum drug concentration (c) and the time (t) that an effective level is maintained ($c \times t$) at the tumor site to success or failure in treating L1210. These studies and those of Wodinsky et al. (*28*), with the L1210 spleen colony assay modified after the AKR leukemia assay (*29*) plus empirical schedule-dependency tests in leukemic and normal animals (*30–38*), show that the most effective treatment schedule is one that exerts maximum selective toxicity against tumor cells relative to sensitive normal cells. Thus the $c \times t$ at the site of critical normal cell toxicity and the proliferative state of the normal cells are added factors in the efficacy of cell-cycle, stage-specific drugs.

Many years of studying empirical treatment schedules have not produced "one best schedule" for all drugs or even two best schedules (one for DNA inhibitors and one for other drugs), probably because of the simple (but not universally appreciated) point that drugs vary with respect to the influence of the treatment schedule on relative toxicity to tumor and normal host cells. For example, it might be assumed that prolonged maintenance of a high concentration of any active drug would result in maximum effect regardless of the mechanism of drug action, because each viable tumor cell eventually enters a sensitive stage of its life cycle or attempts to carry out a critical metabolic reaction. Such schedules have been successful in the chemotherapy of bacterial infections when the drug kills the infecting microorganism by inhibiting a biochemical pathway of no importance to the mammalian host cells. Consequently, bactericide concentrations in the body are not toxic to the host. In contrast, prolonged infusions or intensive uninterrupted treatment of tumor-bearing animals with active drugs (e.g., treatment every 3 hr for 5 consecutive days) have generally reduced therapeutic effectiveness (Drug Research and Development, DCT, NCI: Unpublished data). This circumstance is not surprising in view of the similarity of the basic intracellular events leading to proliferation of normal and malignant cells.

TABLE 8.—*Cell-cycle parameters of some experimental tumors*

Tumor	Tumor size	TC, hr	TS, hr	TS/TC	PF, %	TD, days	References
L1210-ip (ascites), mouse	10^6 cells	12.8	9.0	0.07	86	0.5	(*11, 12, 15–17*)
B16-ip (solid), mouse	5–10 mm	15.2	8.2	—	50	2.7	(*13*)
B16-sc (solid), mouse	560 mg	20.0	7.0	0.35	55	1.9	(*11, 12, 15*)
AK-spontaneous leukemia, mouse	625 mg (thymus)	14.5	8.0	0.55	—	0.5	(*15*)
LL-sc (solid), mouse	575 mg	19.0	8.5	0.45	38	2.9	(*11, 14, 15*)
DMBA-induced, rat	440 mg	18.0	9.5	0.53	10	7.4	(*11, 15*)

The early years of the Chemotherapy Program were largely devoted to the considerable task of developing the resources needed to conduct primary antitumor screening of unprecedented proportions (*39*). Because of suggestive evidence of predictive value for the clinic (*40*), L1210 leukemia was selected as one of the in vivo tumors and has been used continuously as a primary screen in the program. Other early screens (S180, Ca755) were selected because their cytology and pathology resembled major human malignant disease and the mice required could be readily obtained. Outbred mice could be used for S180 and BDF_1 animals for both L1210 and Ca755 screening (*39*).

The significant effect of treatment schedules on the response to active drugs was noted early in the program (*32*), and compounds active in the L1210 screen were subjected regularly to treatment schedule studies. The schedules generally included treatment twice a day (usually morning and afternoon), 1 day only, once daily, every second or third day, and weekly. More recently, the concepts relating cell kinetics and chemotherapy (*41, 42*) have prompted intensive study of this relationship for L1210 (*43, 44*). Studies of the kinetics of proliferation of early L1210 in ascites (*16, 45*) indicate, as mentioned above, that the cell-doubling and cell-cycle times are about one-half day. TS/TC (TS = time of S phase; TC = cell-cycle time) is approximately 0.7, with a growth fraction of greater than 80%. As a result of the quantitative data available on tumor-cell kinetics and the effect of treatment schedules on antitumor activity, the current basic protocol (table 11) (*46*) was established for initial (Type I) schedule-dependency testing of drugs active in the L1210 primary screen. The information gathered in these tests is important in designating schedules for toxicology examination and in developing clinical drug formulations.

In the Type I schedule-dependency test, the single- and daily-treatment schedules are identical to those used for primary screening in recent years. They serve as positive and/or negative controls to confirm screening results and provide internally controlled base-line activity levels for evaluating alternative schedules. Daily treatment is given for 5 and 9 days to assess the extent to which the longer duration, although effective, may be an overtreatment. Retrospective comparison of the 5- and 9-day courses for a large number of drugs aids in selecting the appropriate duration of daily treatment for primary screening, an important factor in efficient use of laboratory resources and available materials. The single rapid injection, if effective, provides an estimate of the percentage of leukemia cells destroyed by a given drug dose. The relative effectiveness of intermittent schedules (every 4 or 8 days) furnishes information on the relative rate of tumor and host recovery after each injection. Intermittent treatments and the single-day treatment are given in divided injections (every 3 hr for 24 hr) as well as one injection on the scheduled days of treatment. These divided intermittent or around-the-clock schedules are a realistic experimental approach to the study of single or widely spaced courses of limited infusion. In addition, treatment over a 24-hour period allows a specific inhibitor of the S phase of the cell cycle to exert its action against the majority of proliferating L1210 cells, since nearly all should traverse S phase twice in each treatment course. Limited schedules of sc injection or oral administration are included since these are not used routinely in primary screening, which at this point of new drug development usually provides data only for the ip route.

In addition to the Type I schedule-dependency test, drugs may be studied further with respect to the influences of route of administration, site of tumor implantation, size of initial tumor inoculum, and extent of disease at treatment initiation, but such tests are not routine because their relative importance

TABLE 9.—*Influence of the site of tumor implantation on drug activity against B16 melanoma*

Drug	Tumor site[a]	
	ip, % ILS	sc or im, % ILS
Adriamycin	200	N
Daunorubicin	160	N
Vinblastine	120	N
Vincristine	89	N
Dactinomycin	103	30
Mitomycin C	67	N
CCNU	200	100
BCNU	109	61
Melphalan	105	N
HN2 (mechlorethamine)	85	N
Cyclophosphamide	62	30
Chlorambucil	32	N
Thio-TEPA (triethylenethiophosphoramide)	N	N
Methotrexate	N	N
5-FU	40	N
5-FUDR (floxuridine)	38	N
6-Mercaptopurine (6-MP)	N	N
Cytosine arabinoside (ara-C)	60	25
Bleomycin	N	68

[a] % ILS = percent increase in life-span over controls. N = ILS < 25%.

TABLE 10.—*Activity of bleomycin (NSC-125066)[a] in mice bearing implanted B16 melanoma[b]*

Dose, mg/kg/injection	Tumor implantation method							
	ip		im		sc		ic	
	MST[c]	% ILS[d]	MST[c]	% ILS[d]	MST[c]	% ILS[d]	MST[c]	% ILS[d]
0	22.5	0	24.0	0	22.5	0	15.0	0
4	24.0	6	32.5	35	37.0	64	16.5	10
8	23.0	2	36.5	52	37.0	64	18.0	20
16	25.0	11	37.0	54	38.0	68	16.0	6
32	11.0	-52	11.0	-54	7.0	-69	8.5	-44

[a] Drug administered ip.
[b] Data of Wodinsky et al. (*28*).
[c] MST = median survival time in days.
[d] % ILS = percent increase in life-span over controls.

TABLE 11.—*Protocol for initial (Type I) schedule-dependency study against L1210* [a]

Drug route	Treatment schedule [b]
ip, sc, oral	Once on day 1 only
ip	Every 3 hr on day 1 only
ip	Once daily, days 1–5
ip, sc, oral	Once daily, days 1–9
ip	Once every 4th day, days 1, 5, and 9
ip	Every 3 hr, every 4th day, days 1, 5, and 9
ip	Once every 8th day, days 1 and 9
ip	Every 3 hr, every 9th day, days 1 and 9

[a] The experimental method for the Type I L1210 schedule-dependency test is generally that used for primary screening and modified as follows. Each treatment schedule includes 4 or 5 dose levels, ranging from an ineffective or minimum effective dose to a frankly lethal dose, given to parallel groups of 8–10 leukemic mice and 6–8 normal mice. For each schedule, as the dose is increased, the survival time of leukemic mice is increased until host toxicity becomes limiting. A further increase diminishes survival time. Relative effectiveness among schedules is based on the maximum increase in life-span of leukemic mice over controls provided by the optimum dose or the highest nonlethal dose. In some studies at the Southern Research Institute (Laboratory 08), a similar but modified protocol uses treatment on day 2 only (single injection and every 3 hr), intermittent treatment on days 2, 6, 10, and 14 (one treatment on days 2–16). Results for drugs examined by both protocols have not differed significantly. *See* (*46*).

[b] Day 0 = day of ip leukemic implant (10^5 cells/mouse).

depends on the results of the Type I study and on data from other areas of the Chemotherapy Program. For example, the need for detailed schedule-dependency tests involving oral administration with an orally and parenterally active drug may depend on the prognosis for developing a parenteral clinical formulation. For drugs without oral activity, varying the site of tumor implantation and parenteral drug administration provides information on antitumor activity when the drug is subjected to the pharmacologic and metabolic hazards normally encountered in vivo.

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Toxicology of Anticancer Drugs

Anthony M. Guarino[1]

After a potentially useful chemotherapeutic agent has been selected through the drug evaluation procedures described earlier in this monograph, precise knowledge of its toxicologic potential must be obtained by systematic studies in laboratory animals. This stage of new drug development corresponds to stage III in the linear array.

Here questions must be answered regarding the highest nontoxic dose of the drug in dogs and monkeys, the nature of the toxicity, the major organ toxicity in two species, the predictability of toxicity, dose–response effects, manageability or reversibility of toxicity, quantitative and qualitative consistency of findings within species, and the overall adequacy of these data. When these questions are answered, it is possible to make qualitative and quantitative comparisons of the consistency of results between species.

Before filing an investigational new drug application, the preclinical toxicology is summarized to show tolerated doses in milligrams per kilogram and milligrams per square meter for each species, schedule, and route. Recommendations are made for an initial clinical dose and warnings of potential toxic effects are stated.

To obtain this needed information, we use a standard protocol (Prieur et al., this monograph, appendix IV), which includes the following studies: 1) single dose (dogs); 2) five consecutive daily treatments (dogs); 3) five consecutive daily treatments (monkeys); 4) five consecutive daily treatments (dogs), 9-day rest, repeated for three treatment periods; and 5) schedule-dependency studies (dogs), with either *a*) 48-hour infusion weekly for 6 weeks, *b*) treatment every 6 hours for 48 hours for 6 weeks, *c*) weekly treatment for 6 weeks, or *d*) 10 consecutive daily treatments.

The single-dose schedule provides a pilot study to select an initial dose level for subsequent studies in dogs and monkeys and acquaints the investigator with the general toxicologic potential of the drug being tested. The 5-day dose treatment in dogs determines the toxicologic parameters encountered with repeated administration of graded doses of the compound for comparison with the results of single treatment. The same treatment in monkeys provides information concerning possible species differences of drug effects. The 5-day treatment followed by a 9-day rest, repeated for three treatments, allows an estimate of the rate of recovery from drug toxicity and helps to determine cumulative drug effects.

Forty-eight-hour infusions or treatments every 6 hours are selected for drugs known to have schedule dependency in experimental tumor systems, and they are intended to duplicate those to be used in phase I clinical trials. Weekly drug treatment is studied when this schedule is likely to be used clinically.

Each study group routinely includes male and female animals. Half the animals are killed 1 day to 1 week after the last treatment, whereas the rest are allowed at least 45 days to recover from drug effects or to show signs of delayed toxicity.

Ophthalmoscopic examinations and clinical tests are performed once 8–10 days before and once within 3 days before starting drug treatment to establish pretreatment values needed for comparison with posttreatment results. After drug treatment, animals are observed daily for clinical signs of toxicity and a record of food and water intake is maintained during the test period. Body temperature also is recorded before and after drug treatment. A full set of hematologic and chemical parameters are determined each week after drug treatment, and after the animals are killed, tissues are evaluated for gross and microscopic lesions.

Some of the routine hematologic tests are hemoglobin, hematocrit, erythrocyte and reticulocyte counts, white blood cell count, differential white cell count, platelet count, and bone marrow histopathologic evaluation. The routine clinical chemistry tests are serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), bilirubin (total and direct), sulfobromophthalein sodium retention (BSP), alkaline phosphatase, prothrombin time, blood glucose, blood urea nitrogen (BUN), serum creatinine, and sodium, potassium, calcium, chloride, and magnesium. Routine urinalysis includes specific gravity, pH, glucose, and microscopic cell count (red blood cells, white blood cells, and casts).

Drug effects on the bone marrow are indicated by the hematologic tests, and lymphoid changes are suggested by alterations in lymphocytes and lymph nodes or histopathologic lesions of splenic lymph follicles. Gastrointestinal toxicity is indicated by weight loss, nausea, vomiting, diarrhea, constipation, stomatitis, ulcerations, and histopathologic changes of the esophagus, stomach, or intestine.

Hepatic-biliary toxicity is suggested by increased BSP retention, elevated alkaline phosphatase or transaminases, elevated bilirubin, altered prothrombin time, and histopathologic changes of liver or gall bladder or both. Renal toxicity is evidenced by polyuria, proteinuria, increased BUN and creatinine levels, changes in serum electrolytes, urinalysis, and histopathologic lesions of the kidney, urinary bladder and/or ureter. Neurologic or muscular toxicity generally is indicated by observing the animal for abnormal signs or loss of reflexes. Histologic examination of the eye or optic nerve, spinal cord, peripheral nerves, and skeletal muscle also is valuable. Cardiovascular effects are seen as flushing, hypotension or hypertension, edema, arrhythmias, change in heart rate, and cardiac histopathologic changes. Respiratory problems may be dyspnea, cough, bronchospasm, hemoptysis, and histopathologic pulmonary lesions.

Drug doses are increased or decreased geometrically until a range of doses, from lethal to nontoxic levels, are established. The clinical parameters described indicate the major organ toxicity, which might be confirmed by histopathologic evaluation. The dose–response relationship of the toxicity helps to define further the predictability of the toxic hazard.

The retrospective evaluation of anticancer drugs in dogs and monkeys for the prediction of qualitative toxic effects in man,

[1] Chief, Laboratory of Toxicology, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014.

to be discussed later in this paper, points out the necessity for using two large animal species to eliminate the vast number of false negative predictions that would occur if either species were used alone. Therefore, studies are performed initially with dogs and monkeys. The importance of dosage schedule to drug toxicity is indicated by the difference in toxicity recorded when the drug is administered by various regimens. Finally, an attempt is made to use the data gathered to describe the sequence of biochemical or pathophysiologic events in the drug-induced disease, but it is in this area that the least is known. There is a vast difference in knowing that a dog with elevated BUN levels died 80 days after receiving a single dose of a drug and actually understanding the basic biochemical changes involved in this manifestation of toxicity. Of course, this is also the case with the mechanism of drug action. Too often, all that is known is the overall drug effect rather than its mechanism of action. Such deficiencies in our knowledge make it difficult to develop "rescue" or other antidotal techniques.

With the toxicity data that have been gathered and analyzed, an attempt is made to estimate a safe, starting clinical dose to warn of the possible toxic effects that will be encountered clinically, indicate the order of development of the warning signs of these toxic effects, describe possible delayed hazards, and to indicate the reversibility of any toxic effect.

The detailed procedures for clinical toxicologic evaluation of cancer chemotherapeutic agents of the Laboratory of Toxicology are included in this monograph as appendix IV.

In preclinical toxicology studies, the National Cancer Institute (NCI) prefers to describe drug dosage in milligrams per square meter. The square meter concept merely states that the toxicity of anticancer agents can be more closely correlated between species in terms of dose per square meter of body surface than in milligrams per kilogram body weight. The idea that the usual doses of certain anticancer drugs in various animal species and man are comparable when measured in milligrams per square meter is not new and was first suggested by Pinkel (*1*). Body surface area is also the basis for correlating a variety of physiologic parameters.

Toxicity data from small animals (mice, rats, and hamsters) and large animals (dogs and monkeys) have been gathered, placed on a reasonably similar basis, and compared quantitatively (*2*). Each animal species and all species combined were used to predict the toxic dose of a drug in man. On a milligram per kilogram basis the maximum tolerated dose (MTD) in man can be equated to the LD10 (lethal dose for 10% of the treated animals) by the formula:

$$\text{MTD in man (milligrams/kilogram)} = (\text{km})_i \times (\text{dose in milligrams/kilogram})$$

where $(\text{km})_i$ is the appropriate factor for converting MTD's in animals to those in man. On a milligram per kilogram basis, the MTD in man is about one-twelfth the LD10 in mice, one-ninth the LD10 in hamsters, one-seventh the LD10 in rats, one-third the MTD in rhesus monkeys, and one-half the MTD in dogs. In terms of milligrams per square meter, however, the MTD in man is about the same as that in each animal species.

Thus relationships among the various animal species and man are simpler and more direct with the body surface concept. The results support the conclusion that the experimental test systems for evaluating toxic effects of potential anticancer agents correlate closely with the results in man. There is no doubt that the milligrams per square meter conversion is of value in estimating doses in large animals, including man, when extrapolating from rodent toxicity. However, in everyday terms, when working with dogs and monkeys, there is little difference in predictability whether one uses milligrams per square meter or milligrams per kilogram. This fact does not in any way invalidate the latter conversion concept but most likely reflects the relatively small conversion factors (2 for dogs and 3 for monkeys).

LARGE ANIMAL TOXICOLOGY IN THE QUALITATIVE AND QUANTITATIVE PREDICTION OF DRUG TOXICITY IN MAN

Studies of large animal toxicology provide both a qualitative and quantitative prediction of the human experience. The qualitative aspects highlight the organ systems most likely to undergo damage and determine whether the toxic effects will be predictable, treatable, and reversible. The quantitative information entails the choice of an initial dose for clinical trial and a prediction of the steepness of the dose–response curve.

Qualitative Approach

One of the most controversial areas in the development of new drugs for use in man involves the efficacy of animal toxicology as a predictive system for qualitative toxicity. The general approach of evaluating a new compound for safety in several animal species is performed in an attempt to generate information that may alert the clinical pharmacologist to potential hazards. The process requires extrapolation of toxicity data from one species to another, and implicit is the assumption that certain animal species have significant predictive value for toxicity in man and that important toxicity will not go unpredicted.

Schein et al. (*3*) reviewed the available data on 25 compounds of diverse chemical and functional classification in an attempt to see how well the combined dog and monkey screen predicted qualitative toxicities in man. These drugs included commonly used and well-known compounds such as cytosine arabinoside, 5-fluorouracil, daunorubicin, mitomycin C, mithramycin, DTIC, and BCNU, as well as lesser known compounds like dibromomannitol, streptozotocin, porfiromycin, hexamethylmelamine, azotomycin, and 1-acetyl-2-picolinoyl hydrazine.

Table 1 outlines the method of data analysis and the percentage of the 25 drugs producing individual parameters of toxicities. A greater percentage of the drugs produced toxicity in animals than in man.

The combination of dog and monkey as a toxicologic screen predicted myelosuppression by each of the 22 compounds that produced this effect in man. The total lack of false negatives, or underprediction, was the result of an overlap in information contributed by the use of the two species. The success is not so complete when individual parameters of myelosuppression are considered.

The combined animal screen predicted all drugs that produced anemia but with a 44% overprediction. The monkey and the dog performed equally well in predicting leukopenia with a 68% combined true positive. However, false negative results, or underprediction that was not corrected by the combination, were found for both species. Table 2 lists those compounds in which one or both animal species failed to predict leukopenia. There are only two compounds for which both the dog and monkey failed to predict leukopenia, i.e., the progestational agent (NSC-17256E) and the antibiotic (NSC-56408). Both caused only a slight incidence of leukopenia in man, none of it severe. Because of the manner in which the program for evaluation was written, any compound producing a toxic parameter in any patient caused that parameter to be listed as positive in man, even if the incidence was low.

TABLE 1.—*Prediction of qualitative toxicity from laboratory animals to man*[a]

A) Percentage of the 25 drugs producing the indicated toxic signs

Toxic sign	Dog	Monkey	Man
Anemia	80	83	48
Leukopenia	72	74	80
Thrombocytopenia	72	30	76
Vomiting	96	30	84
Diarrhea	84	30	40
Elevated alkaline phosphatase	76	24	32
Elevated SGOT	60	56	44
Azotemia	64	65	36
Convulsions	20	0	4
Ataxia	44	17	12

B) Method of data analysis

Result	Animal	Man
True positive	+	+
False positive (overprediction)	+	-
True negative	-	-
False negative (underprediction)	-	+

[a] Data adapted from Schein et al. (*3*).

Thrombocytopenia was correctly predicted for 13 of the 18 compounds causing this toxic effect in man. The compounds for which thrombocytopenia was not predicted also are given in table 2. The most serious failure was with mithramycin (NSC-24559), which can cause severe thrombocytopenia. NSC-69945, a cyclophosphamide analogue, shares the platelet-sparing effect of its parent compound but did cause platelet depression in a few patients.

Before considering the information contained in tables 3–5, their format should be explained. In each case, we desired to test whether the appearance of an easily observable sign (e.g., in table 3), such as vomiting or diarrhea, was as predictive of human toxicity as was a composite assessment of toxicity (e.g., in table 3), composite gastrointestinal toxicity as measured by recording incidences of vomiting, diarrhea and other parameters such as the following: weight loss, hematochezia, as well as gross and histopathologic lesions throughout the gastrointestinal tract.

The combined dog and monkey screen gave 92% true positives and no false negatives for gastrointestinal toxicity when compared with that of man.

The prediction of gastrointestinal toxicity is outlined in table 3; vomiting was the manifestation most consistently induced by drugs and it occurred with 18 of the 25 compounds. The

TABLE 2.—*Compounds for which one or both animal species failed to predict leukopenia and thrombocytopenia*[a]

Compounds	Species that failed to predict for leukopenia in man	Species that failed to predict for thrombocytopenia in man
NSC-13875: hexamethylmelamine	Monkey	Monkey
NSC-17256E: pregn-4-ene-3,11,20-trione 6α-methyl	Dog, monkey	—
NSC-19893: 5-fluorouracil	—	Monkey
NSC-24559: mithramycin	Monkey	Dog, monkey
NSC-26980: mitomycin C	—	Monkey
NSC-40774: 9*H*-purine,6-(methylthio)-9-β-D ribofuranosyl-, dihydrate	Dog	Monkey (not measured in dog)
NSC-51095: ammonium, trimethylpurin-6-yl-chloride	Monkey	Monkey
NSC-52947: pactamycin	—	Monkey
NSC-53398: restrictocin	—	Dog, monkey
NSC-56408: tubercidin	Dog, monkey	—
NSC-62512: acetophenone, 2-(dimethylamino)-3′,4′-dihydroxy-hydrochloride	—	Dog, monkey
NSC-65346: sangivamycin	Dog	—
NSC-69945: phosphorodiamidic acid, *N*,*N*,-bis(2-chloroethyl)-, compounded with cyclohexylamine (1:1)	—	Dog, monkey

[a] Adapted from Schein et al. (*3*).

TABLE 3.—*Prediction of gastrointestinal toxicity*[a]

Sign	True positive, %	False positive, %	True negative, %	False negative, %
Vomiting				
Dogs	72	16	0	12
Monkeys	26	13	4	57
Dogs and monkeys	72	16	0	12
Diarrhea				
Dogs	36	40	20	4
Monkeys	13	26	35	26
Dogs and monkeys	36	44	16	4
Composite gastrointestinal toxicity[b]				
Dogs	92	8	0	0
Monkeys	74	9	0	17
Dogs and monkeys	92	8	0	0

[a] Adapted from Schein (*4*).

[b] Composite toxicity in this system was assessed by recording incidences of vomiting and diarrhea, as well as the following parameters: weight loss, hematochezia, gross and histopathologic lesions of the gastrointestinal tract.

TABLE 4.—*Prediction of renal toxicity*[a]

Sign	True positive, %	False positive, %	True negative, %	False negative, %
BUN elevation				
Dog	24	36	28	12
Monkey	18	46	18	18
Dog and monkey	24	52	12	12
Proteinuria				
Dog	9	73	18	0
Monkey	0	43	43	14
Dog and monkey	9	73	18	0
Composite renal toxicity[b]				
Dog	32	56	4	8
Monkey	35	43	13	4
Dog and monkey	36	56	4	4

[a] Adapted from Schein (4).
[b] Composite toxicity in this system was assessed by recording the following incidences of elevated BUN values, proteinuria, as well as the following parameters: polyuria, polydypsia, dysuria, hematuria, gross and histopathologic lesions of the urinary system.

TABLE 5.—*Prediction of liver toxicity*[a]

Sign	True positive, %	False positive, %	True negative, %	False negative, %
Increased BSP retention				
Dog	28	50	5	17
Monkey	33	45	11	11
Dog and monkey	26	47	5	21
Elevated alkaline phosphatase				
Dog	33	42	17	8
Monkey	15	10	40	35
Dog and monkey	32	50	16	12
Elevated SGOT				
Dog	28	39	22	11
Monkey	33	23	33	11
Dog and monkey	35	40	20	5
Composite liver toxicity[b]				
Dog	52	44	4	0
Monkey	52	35	13	0
Dog and monkey	52	48	0	0

[a] Adapted from Schein (4).
[b] Composite toxicity in this system was assessed by recording incidences of increased BSP retention, elevated alkaline phosphatase, elevated SGOT, as well as the following parameters: increased serum bilirubin or globulin, decreased serum albumin, gross and histopathologic lesions of the liver and biliary systems.

combined screen gave 72% true positives but missed 12% of the drug-related emesis. These results were primarily contributed by the dog, since there was significant underprediction in the monkey. Almost the same situation was true for diarrhea.

The large animal screen gave 36% true positives for renal toxicity, as outlined in table 4. Underprediction occurred in only 1 (4%) of 25 compounds, but there was a 56% overprediction. In general, no specific advantage could be attributed to use of either the dog or monkey. BUN elevation and proteinuria gave a high degree of overprediction. The high incidence of renal function abnormalities in animals had its counterpart in the renal histopathologic changes that were documented in most animals.

Table 5 includes the prediction of hepatotoxic effects. The combined screen predicted all instances of hepatotoxicity but at the expense of a 48% overprediction. The comparison of dog and monkey failed to disclose a species advantage. When individual parameters were analyzed, the transaminase and alkaline phosphatase proved to be the most useful.

This study, which is reported more fully elsewhere (3), indicates that use of dogs and monkeys is essential to adequate prediction. It is clear that toxicologic data collected in both species can forewarn the clinician of an effective proportion of the total spectrum of organ-specific and, with certain stated limitations, specific parameter toxicities that might be encountered. With the possible exception of central nervous system and dermal toxicity, all serious organ system toxicities are well predicted. This prediction level is accomplished in many instances at the expense of a high percentage of false positive predictions, but it is believed that this degree of inefficiency in the system is justifiable. For all possible qualitative toxicities to be demonstrated, the animals must receive a dose spectrum including highly toxic and lethal doses. If the clinical use of the drugs in this analysis had been at more toxic dose levels than the estimated MTD, the frequency of false positive predictions by the animal species might have been significantly lower.

It is believed that the effective use of animal toxicologic data coupled with careful monitoring, considered judgment, and

expectation by the physician jointly serve to forewarn of the development of target organ system toxicities during an initial phase I trial.

Quantitative Approach

The great difficulties inherent in the extrapolation to man of results obtained in animal studies have been recognized for many years. Since its inception, the Drug Development Program of the Division of Cancer Treatment (DCT), NCI, has grappled with these problems.

In 1966, Freireich et al. (*2*) first discussed the quantitative comparison of the toxic effects of 18 selected anticancer agents in mouse, rat, hamster, dog, monkey, and man. They found that the MTD in man, in milligrams per square meter was about the same as that in each animal species. On the basis of milligrams per kilogram, the MTD in man was one-third that in rhesus monkeys and one-half the MTD in dogs. The largest ratio of predicted dose/observed dose was 3:1 (thio-TEPA) of the 18 agents. Freireich et al. concluded that it would be reasonable to estimate the human MTD (milligrams per square meter) from the preclinical toxicology in the mouse, rat, dog, monkey, and hamster, and to start clinical cancer chemotherapy trials at about one-third the predicted dose.

Currently, the DCT Laboratory of Toxicology defines four dose levels of toxicity in large animals:

Highest nontoxic dose (HNTD).—The highest dose at which no hematologic, chemical, clinical, or pathologic drug-induced alterations occur. Doubling this dose produces the aforementioned alterations.

Toxic dose low (TDL).—The lowest dose to produce drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters. Doubling this dose *produces no lethality.*

Toxic dose high (TDH).—The lowest dose to produce drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters. Doubling this dose *produces lethality.*

Lethal dose (LD).—The lowest dose to produce drug-induced death in any animals during the treatment or observation period.

In the choice of an initial dose for beginning phase I studies in man, DCT employs one-third the TDL (on a milligrams per square meter basis) observed in the most sensitive species.

Recently, it was considered worthwhile to evaluate the quantitative interspecies comparison among dogs, monkeys, and man for a series of 15 agents, which recently completed clinical trials, to determine the actual predictability of the new dose levels described above. Although such a comparison clearly might not work for every drug, because of interspecies differences in pharmacologic disposition, it probably works often enough to make the analysis valuable.

All the compounds chosen for study (table 6) entered clinical trial within the last several years. Data gathered from completed protocol studies of the Laboratory of Toxicology were searched in an attempt to identify the four defined dose levels. An effort was always made to translate the dose schedules in the animals to the identical one used in man when the schedules were not identical. It was assumed that the toxicity of anticancer agents is cumulative, as Griswold et al. (*5*) showed in their work with mice and also accepted in the work of Freireich and his co-workers (*2*). For example, a dose of 10 mg/m^2/day $\times$ 5 in animals translated to 5 mg/m^2 $\times$ 10 or 1 mg/m^2/day $\times$ 50.

The human MTD (milligrams per square meter) of each drug was compared on this same basis when possible to the four dose levels in the beagle and rhesus monkey described above. The quantitative comparison was made by determining the ratio of the human MTD to the various animal dose levels. Thus if the ratio of the human MTD to a given animal dose is more than 1, the human MTD dose is higher than the animal dose, and man would be the more sensitive species, if the dose levels studied were considered comparable.

An example of the analysis for CCNU (NSC-79037) is shown in table 7. The ratio of the human MTD to the TDL is 3.25 in dogs and 3.6 in monkeys, and man is apparently the less sensitive species at those dose levels. The initial dose chosen for clinical trial was one-third the TDL (15 mg/m^2) and the NCI–Veterans Administration (VA) Medical Oncology Service Phase I Study reached the human MTD in six steps with a modified Fibonacci search scheme for dose escalation.

Table 8 outlines the ratios obtained for the 15 drugs in the beagle. In addition, the LD/TDL ratio in the beagle is included as a possible representation of the steepness of the dose–response curve. The last column of the table shows the number of steps it would have taken in a modified Fibonacci approach to reach the established MTD in man starting with one-third the TDL in the beagle. These data could also be considered a representation of the predictive value of the beagle for man.

When the ratio of the human MTD to the beagle TDL shown in table 8 is considered in detail (table 9), it is seen that three drugs (camptothecin, phenesterin, and pseudourea) have a ratio less than 1, which means that, if the choice of the initial

TABLE 6.—*Drugs used in quantitative interspecies comparison*

NSC number	Common name or abbreviation	Chemical name
1895	Guanazole	
18429	Aniline mustard	*N, N*-Bis(2-chloroethyl) aniline
45388	DTIC, ICT, DIC	Imidazole-4 (or 5)-carboxamide, 5(or 4)-3,3-dimethyl-1-triazeno)
56054	Pseudourea	2,2′-(9,10-Anthrylenedimethylene)bis(2-thio-thiopseudourea), dihydrochloride, dihydrate
71261	β-TGDR	β-2′-Deoxythioguanosine
79037	CCNU	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
82196	TIC mustard	Imidazole-4 (or 5)-carboxamide, 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]-
85998	Streptozotocin	
100880	Camptothecin sodium	
102816	5-Azacytidine	
104469	Phenesterin	
104800	Dibromodulcitol	
107392	5-HP	5-hydroxy-picolinaldehyde, thiosemicarbazone
406021	Ara-6-mercaptopurine	
409962	BCNU	Urea, 1,3-bis(2-chloroethyl)-1-nitroso-

human dose had been made directly from the beagle TDL, this dose would have been in the clearly unacceptable range of toxicity. This 20% rate of failure of the beagle TDL for predicting an initial nontoxic dose when translating directly means that this approach would not be feasible. Nine drugs had a ratio between 1 and 10, and three had a ratio more than 10. The mean and median ratios for the 15 drugs are 6.5 and 3.4, respectively, with a range of 0.3–25.0.

When one-third the TDL in beagles is used to determine the ratio with human MTD, the situation improves considerably (table 10). Only one drug (camptothecin) has a ratio of less than 1; when studied in detail, it is only less than 1 by one of the three schedules (table 11) for which it is possible to make the analysis. The phase I trial at the NCI Baltimore Cancer Research Center was started cautiously at a much lower dose (18 mg/m^2), and thus serious toxicity in the early study was avoided. The analysis depicted in table 10 gives us confidence that one-third the TDL in the most sensitive animal species is a safe approach, although it cannot be considered "absolutely" safe.

When the ratio of human MTD to TDH of the beagle is examined (table 12), all ratios are close to 1. Five drugs have a ratio greater than 1, whereas in eight (62%), it is less than 1, which indicates the TDH would not be acceptable for direct translation to a safe initial dose for more than 50% of the time. If it is assumed that the TDH in animals is the MTD, then man is a more sensitive species for more than half the time.

Data in the rhesus monkey are available for only six drugs (table 13), which limits any meaningful comparisons. The ratio of the human MTD to the one-third TDL is more than 1 for all drugs tested, whereas the ratio of human MTD to monkey TDL is less than 1 for three of five drugs; again the danger of using the TDL without fractionating it in the translation to man is emphasized.

TABLE 7.—*Quantitative interspecies comparison for single oral doses of CCNU*

Dose level in animals	Dog		Monkey	
	mg/m^2	Human MTD animal dose[a]	mg/m^2	Human MTD animal dose
HNTD	—	—	—	—
TDL	40	3.25	36	3.6
TDH	—	—	—	—
LD	200	0.65	—	—

[a] Human MTD = 130 mg/m^2; NCI-VA phase I study used a modified Fibonacci search scheme to escalate from 15 mg/m^2 to 130 mg/m^2 in six steps.

TABLE 8.—*Ratios of human clinical doses to various doses*

NSC number	Drug name or abbreviation	Ratio of human MTD to dose levels in beagle				Beagle LD/beagle TDL	Estimated number of Fibonacci steps
		TDL	⅓ TDL	TDH	LD		
1895	Guanazole	2	6	0.6	0.3	6	5
18429	Aniline mustard	5	15	0.34	0.17	30	8
45388	DTIC	1.6	4.8	0.8	0.4	4	4
56054	Pseudourea	0.4	1.2	0.28	0.14	3	4
71261	β-TGDR	14	42	1.7	0.8	16	12
79037	CCNU	3.2	9.6	—	0.65	5	6
82196	TIC mustard	3	9	1.5	0.75	4	4
85998	Streptozotocin	6.2	18.6	2.5	1.25	4	10
100880	Camptothecin sodium:						
	Single dose	0.3	0.9	0.1	0.07	4.5	0
	Weekly	0.8	2.3	0.17	0.08	10	4
	Daily × 5	1.6	4.8	0.37	0.2	8	11
102816	5-Azacytidine	14	42	3.2	1.8	8	12
104469	Phenesterin	0.8	2.4	0.04	0.02	33	2
104800	Dibromodulcitol	3.4	10.2	0.9	0.45	8	6
107392	5-HP	5.7	17.1	0.65	0.3	18	9
406021	Ara-6-mercaptopurine	25	75	1.8	0.84	30	13
409962	BCNU	10	30		2.5	4	11

TABLE 9.—*Comparison of the ratio of the human MTD to the TDL in the beagle for 15 anticancer agents*

Range of ratios[a]	Number of drugs	Percentage of drugs
0.1–1	3	20
1–10	9	60
>10	3	20

[a] Mean ratio = 6.5; median ratio = 3.4; range = 0.3–25.0.

TABLE 10.—*Comparison of the ratio of the human MTD to one-third TDL in the beagle for 15 anticancer agents*[a]

Range of ratios	Number of drugs	Percentage of drugs	Mean	Median
0.1–1	1	7.0	0.9	0.9
1–10	7	46.5	4.3	4.8
>10	7	46.5	36.0	18.6

[a] Overall mean = 18.7; overall median = 9.6.

TABLE 11.—*Quantitative interspecies comparison for camptothecin administered by three dose schedules*

Dose levels in animals	Single dose (human MTD = 120 mg/m²)		Weekly dose (human MTD = 67 mg/m²)		Daily × 5 (human MTD = 15 mg/m²)	
	Dog mg/m²	Ratio human MTD	Dog mg/m²	Ratio human MTD	Dog mg/m²	Ratio human MTD
TDL	375[a]	0.32	80[b]	0.84	9[c]	1.6
TDH	1,200	0.1	400	0.17	40	0.37
LD	1,700	0.07	—	—	75	0.2

[a] ⅓ TDL = 125 mg/m² and is toxic. (Actual Baltimore Cancer Research Center starting dose was 18 mg/m².)
[b] ⅓ TDL = 27 mg/m². Four steps in modified Fibonacci approach.
[c] ⅓ TDL = mg/m². ≈11 steps in modified Fibonacci (NCI-VA started at 1.5 mg/m² and took 12 steps).

TABLE 12.—*Comparison of the ratio of the human MTD to the TDH in the beagle for 13 anticancer agents*

Range of ratios[a]	Number of drugs	Percentage of drugs
>1	5	38
<1	8	62

[a] Mean = 1.03; median = 0.8; range = 0.04-3.2.

velopment program, but also because we recognize a greater debt to the scientific and medical community, due to the agents with which we work. These anticancer drugs are among the most toxic xenobiotics to which man is exposed. Thus with a spectrum of species treated with this class of agents, as well as human exposure, we owe it to our colleagues working with nondrug xenobiotics to develop the best analytic, methodologic, and statistical predictors of xenobiotic toxicity to man.

TABLE 13.—*Ratios of the human MTD to various dose levels in the rhesus monkey for six anticancer agents*

NSC number	Drug	Ratio of human MTD to dose levels in monkey				Monkey LD/monkey TDL
		TDL	⅓ TDL	TDH	LD	
18429	Aniline mustard	—	—	—	—	8.0
45388	DTIC	0.38	1.14	0.19	0.09	4.0
56054	Pseudourea	0.6	1.8	0.28	0.14	4.0
79037	CCNU	3.6	10.8	—	—	—
85998	Streptozotocin	6.2	18.6	1.7	0.85	7.5
104800	Dibromodulcitol	0.66	1.9	0.15	0.07	9.0
	Mean	2.6	6.6	0.6	0.3	
	Median	0.6	1.9	0.25	0.1	

A comparison of the ratios for the beagle and monkey (table 14) indicates that the monkey is apparently the more sensitive species overall, but the number of drugs tested in the monkey is small and this inference must be made with caution. A more detailed analysis of quantitative comparisons, involving many more drugs and including rodent species, is in progress. Such ongoing studies are important not only to any viable drug de-

TABLE 14.—*Comparison of the ratios of human MTD to various dose levels in the beagle and rhesus monkey*

Ratio	Mean		Median	
	Beagle	Monkey	Beagle	Monkey
MTD/TDL	6.5	2.6	3.4	0.6
MTD/⅓ TDL	18.7	6.6	9.6	1.9
MTD/TDH	1.03	0.6	0.8	0.25
MTD/LD	0.67	0.3	0.3	0.1

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Pharmacology of Anticancer Drugs

Vincent T. Oliverio [1]

Any pharmacologic program, designed to develop clinically useful drugs, has as its objective improved understanding and broader knowledge of species differences in drug disposition, which will greatly improve the projection of the pharmacologic and toxicologic properties of a given drug from experimental animal data to man. In this regard, Mellett's point should be emphasized that it is the quantitative rate of drug disposition rather than its qualitative aspects that is of practical importance in translating drug doses and schedules from animals to the clinic (*1*). Thus knowledge of the quantitative aspects of drug disposition in animals and man and in its interdigitation with other pharmacologic and biochemical knowledge are most important in the drug development program. This is not to negate the importance of the qualitative aspects of drug disposition because there are qualitative differences from species to species. However, the main differences in drug disposition between man and experimental animals are in the rates of drug disposition, biotransformation, and elimination.

One of the early studies published by members of the Subcommittee of the NCI Acute Leukemia Task Force illustrated the quantitative approach to relating host toxicity with drug dosage and scheduling from laboratory animals to man for a number of antitumor drugs (*2*). The conclusion reached was that the lethal dose for 10% of the animals (LD10) in rodents and the maximum tolerated dose (MTD) in the dog or monkey predicted the MTD in man, provided that 1) identical dose schedules, routes, and duration of administration were used in animals and man, and 2) the doses were expressed in milligrams per square meter of body surface rather than in milligrams per kilogram of body weight. In other words, predictability of human toxic effects from animal data was poor by the conventional method of expressing drug dosage. In a sense, this study was the first to establish successfully a quantitative relationship between host toxicity and drug dose levels and schedules, whereby pharmacologic knowledge for use with other quantitative pharmacology to achieve maximum therapeutic benefit from antitumor agents was developed.

At the preclinical level several kinds of studies are needed. Initial studies with animals or in vitro require the development and use of sufficiently sensitive and specific quantitative methods for measuring each individual drug and its metabolites. Several general methods are depicted in table 1. Isotopic tracer or bioassay techniques may be needed in addition to a chemical method. Each method should meet certain criteria on sensitivity, specificity, and reproducibility. When labeled compounds are used, specific activity should be sufficient to meet sensitivity criteria. Radiopurity is of considerable importance, and, of course, the isotopic label, particularly tritium, should not exchange with biologic media. The rapid development of a good analytic technique for estimation of antitumor drugs and their metabolites in biologic media has probably been one of the major bottlenecks in proceeding with pharmacologic disposition studies. Therefore, high priority is given to exploration of the potentialities of newer analytic techniques, such as gas–liquid chromatography, mass spectrometry alone or in combination with gas chromatography, nuclear magnetic resonance, and other physical–chemical techniques, which, in other areas of drug research, have made possible the identification of small amounts of drugs and their metabolites in blood and urine in a manner that was impossible a few years ago. Although this refers to the need for better analytic techniques in preclinical pharmacology, it is obvious that these methods are equally as important to clinical pharmacologic studies in which the identification and quantitation of drugs and their metabolites in blood and urine of patients receiving the usual clinical dose are to be made. Certainly, the assessment of variations in the pharmacokinetic behavior and metabolism of drugs among individuals would not be possible without adequately sensitive and specific analytic techniques.

The second phase of pharmacologic disposition studies at the preclinical level involves application of the analytic techniques to gain information on certain pharmacokinetic variables and on possible drug biotransformations in several animal species after parenteral and oral routes of administration, preferably at LD10 doses. The protocol is outlined as follows:

1) Measure extent of absorption after oral LD10 dose.
2) Measure distribution, excretion, and metabolism by expected clinical route of administration at LD10 dose. Determine:
   a) plasma and tissue concentration × time ($c \times t$): plasma protein binding characteristics
   b) Clearances by kidney, liver (biliary), and gastrointestinal tract
   c) Metabolism: organ, products, rate, biologic activity
3) Conduct related studies on:
   a) Effect of dose schedule on disposition
   b) Drug interactions
   c) Drug effects
   d) Biochemical mechanisms

The animal species usually employed include the mouse, rat, dog, and monkey. If the drug is proposed for oral use in man, the extent of absorption should be measured. The drug should be sufficiently well absorbed to meet certain oral criteria, i.e.,

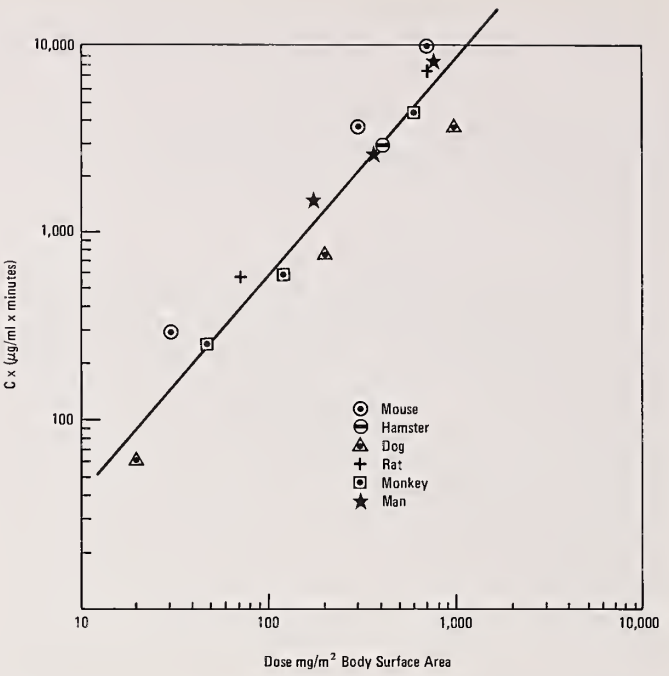
TABLE 1.—*Development of analytic methods for identification and measurement of agents and metabolites*

Methods	Criteria
Chemical	Sensitivity
Bioassay	Specificity
Isotopic	Reproducibility

[1] Associate Director for Experimental Therapeutics, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014.

the area under the plasma curve [concentration × time ($c \times t$)] should be at least 80% of the results after an effective therapeutic parenteral dose. Whether the drug is to be given orally or parenterally to humans, study would then proceed to item 2, the measurement of the distribution, excretion, and metabolism of the agent after the expected clinical route of administration at the LD10 dose, which has the highest priority in a pharmacologic study. After accumulating sufficient data on plasma and tissue drug concentrations as a function of time ($c \times t$), organ clearance rates, and metabolism (as listed under item 2), clinical pharmacologic studies could be started. Then one has to consider collecting information at the preclinical level on the points listed under item 3, but these related studies are not considered urgent for proceeding with the development of the drug for clinical trial. For example, studies on drug interactions or mechanism of action might be conducted during or well after the drug has been in clinical trial. The information gained from these studies might be useful in devising new dose schedules and regimens for more effective therapy.

Part of the philosophy adopted in the pharmacology program is based on the concept that it is the quantitative rate of drug disposition or drug metabolism that will determine plasma and tissue concentrations, and that knowing species differences in metabolic rate may permit extrapolation of data from laboratory animals to man. In other words, just as a relationship was shown between body surface area and dose as a criterion for comparative toxicity or as a means for relating drug doses between the laboratory and clinic, these studies also seek to establish a correlation among the drug dose, body surface area, plasma levels, and plasma half-life of various species as a means for relating a specific pharmacologic response between laboratory animals and man. In particular, plasma drug concentrations in various species are being determined in relation to time after certain dose levels, routes of administration, and dose schedules (the $c \times t$ concept, in which the area under the plasma curve provides a value for interspecies comparison). The data accumulated are then integrated with knowledge of cell-cycle kinetics to arrive at certain treatment schedules for achieving complete tumor cell eradication. This approach was developed largely by Dr. Howard Skipper and his group at Southern Research Institute. Dr. Mellett has found that, for certain antitumor drugs such as cyclophosphamide and methotrexate, an excellent correlation can be made between drug dose (milligrams per square meter) and the plasma $c \times t$ (grams/milliliter × time expressed in minutes) of animals and man (*1*), as illustrated in text-figure 1 and table 2.



TEXT-FIGURE 1.—Relationship between cyclophosphamide dosage (mg/m² body surface area) and $c \times t$ (μg/ml × min) attained in man and animals.

One would expect the type of relationships to occur as depicted in text-figure 1 and table 2 because clearance factors between species, such as biliary excretion, are related to body surface area. This observation especially applies to drugs such as methotrexate which are not significantly metabolized, and for those like cyclophosphamide, which are metabolized. On

TABLE 2.—*Summary of estimated half-lives and integrated* c × t *plasma level values for cyclophosphamide after parenteral administration in various species*

Species	Dose		Route	Half-life (min)	Integrated $c \times t$ (μg/ml × min)
	mg/kg	mg/m²			
BDF_1 mouse	10.0	30.0	ip	17	189
	100.0	300.0	ip	21	3,742
	200.0	600.0	ip	25	10,000
Hamster	100.0	400.0	ip	15	2,907
Dog	1.0	20.0	iv	38	60
	10.0	200.0	iv	32	750
	50.0	1,000.0	iv	32	3,500
Rat	10.0	70.0	ip	43	574
	100.0	700.0	ip	39	7,550
Monkey	4.7	56.4	iv	43	245
	10.0	120.0	iv	47	585
	50.0	600.0	iv	48	4,500
Man	5.0	175.0	iv	210	1,440
	10.0	370.0	iv	216	2,630
	20.0	740.0	iv	198	8,195
	20.0	740.0	iv	195	7,050

the other hand, a lack of correlation between dose, body surface area, plasma levels, and plasma half-life ($T\frac{1}{2}$) is seen for drugs like cytosine arabinoside, which is metabolized by the pyrimidine nucleoside deaminase to uracil arabinoside at varying rates among laboratory animal species and man, because of the difference in the distribution of the enzyme in various tissues among the species studied. Thus there are some exceptions that will interfere with assumptions on which empirical corrections for body size are based.

For many drugs it is difficult to establish a relationship between plasma concentration with time ($c \times t$) and a particular pharmacologic response. These drugs often give low or insignificant plasma concentrations because of extensive tissue distribution. Therefore, it could be important to determine tissue concentration with time. To illustrate this point, the tissue distribution ($c \times t$) of daunorubicin (NSC-82151) and adriamycin (NSC-123127) have been compared in experimental animals in an attempt to understand their different therapeutic indices. This study is being conducted collaboratively by Arthur D. Little, Inc., and the NCI Baltimore Pharmacology Laboratory.

Daunorubicin has clinical effectiveness in the treatment of certain cancers. The closely related antibiotic derivative, adriamycin, has been active against experimental tumors but with a therapeutic-to-toxicity ratio higher than that of daunorubicin. Comparative physiologic disposition studies indicate that both compounds are rapidly cleared from the plasma ($T\frac{1}{2} < 5$ min), deposited in tissues, and slowly excreted into the urine and bile. Daunorubicin, unlike adriamycin, is extensively metabolized. However, the calculated tissue $c \times t$ of the latter is about twice that of daunorubicin plus metabolite. This fact is illustrated in table 3 for several selected organs from treated rats. The data indicate that the time of exposure of tissues to adriamycin can be 60–100% longer than daunorubicin; this increased retention time of the former may correlate with its superior therapeutic index as compared with the latter. Scientists are continuing to consider differences in the pharmacokinetics of these drugs in various host animals and their tumors and to compare their toxicities at similar $c \times t$ values. The data indicate that the metabolism of daunorubicin may further complicate the interpretation of the differences in therapeutic indices.

More recently Zaharko at NCI and Dedrick, a chemical engineer with the Biomedical Engineering Group at the National Institutes of Health, have studied with their colleagues (*3, 4*) the variation of the distribution of methotrexate in mice, rats, dogs, and man at different dose levels and by different routes of administration. The data have been incorporated into a mathematical model to obtain more precise quantitative parameters for predicting drug disposition among species. The parameters that appear to determine the plasma concentration with time of methotrexate at different dose levels and in various species, thus affecting prediction by the model, are shown in text-figure 2. Thus plasma concentration is affected significantly by liver uptake and biliary secretion, gastrointestinal reabsorption and transit, kidney clearance, and muscle. The compartments represented by broken lines may bind the drug but are not believed to play a predominant role in the plasma level.

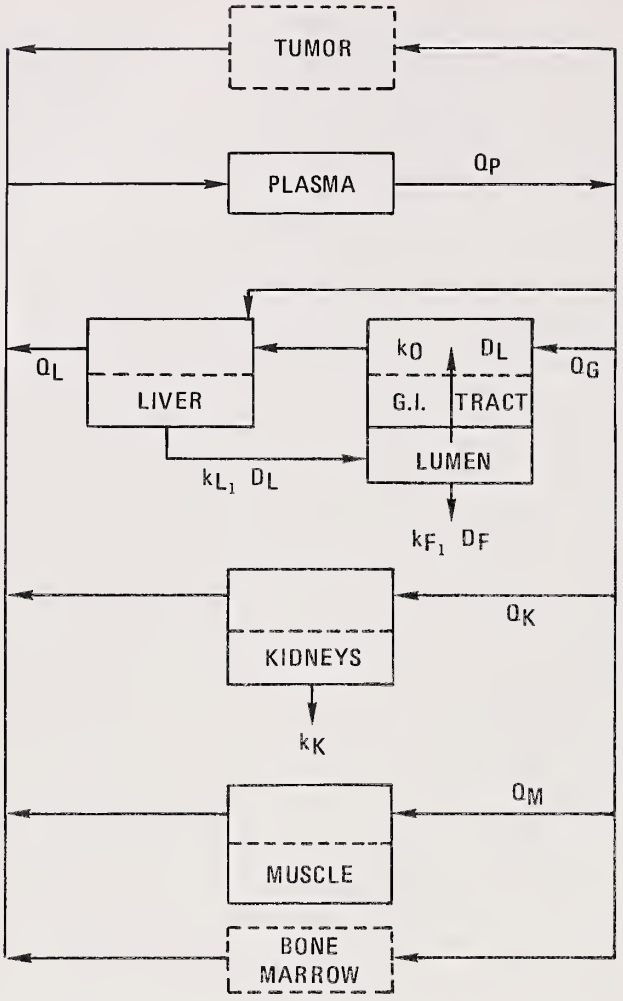
The equations for the mathematical model were based on experimental data representing organ size and blood flow rates, tissue-to-plasma distribution ratios, kidney clearance, biliary secretion, and intestinal absorption. Then by simultaneous solution of the equations with a digital computer, the predicted results agreed with the experimental values. In other words, the drug concentration at any time in any compartment was determined. The predicted values for mice agree with experimental values (text-fig. 3). The model has also predicted reasonably accurately for rats and dogs and for plasma concentrations in man (text-fig. 4). The predicted disappearance curves for muscle, liver, and gut have not been verified experimentally. Dedrick et al. (*4*) introduced the equivalent time concept to explain the species differences in the pharmacokinetic parameters. In simplest terms, this concept states that changes in methotrexate concentrations take place more rapidly in smaller than in larger animals because of differences in organ size, clearance rates, and resident time. The importance of this relatively new approach in studying antitumor drug doses and schedules is apparent when one considers that detailed pharmacologic studies for some drugs may only be required in one species, such as the mouse, to predict pharmacologic disposition in man. In any event, further efforts are being made to extend mathematical modeling to other antitumor drugs to obtain more precise quantitative pharmacologic data with minimal experimentation.

Up to this point, this review has included the pertinent pharmacologic information that should be obtained during the preclinical stage of drug development. Next a limited number of drug disposition studies in man should be considered, ideally during phase I clinical trial. This approach permits maximum use of patient material, minimum expenditure of drug, and early accumulation of pharmacokinetic data in man. An example was a recent NCI pharmacologic and clinical phase I trial of sodium camptothecin (NSC - 100880) conducted collaboratively by the Baltimore Cancer Research Center clinical staff and investigators of the Laboratory of Chemical Pharmacology (*5*).

If preclinical studies have established a relationship between drug plasma concentration and a particular pharmacologic response, then plasma concentration should be determined as a function of dose in man. The most appropriate times for sampling plasma could be determined by referring

TABLE 3.—*Calculated* c × t *of daunorubicin, its metabolite, and adriamycin equivalents in tissues of rats*

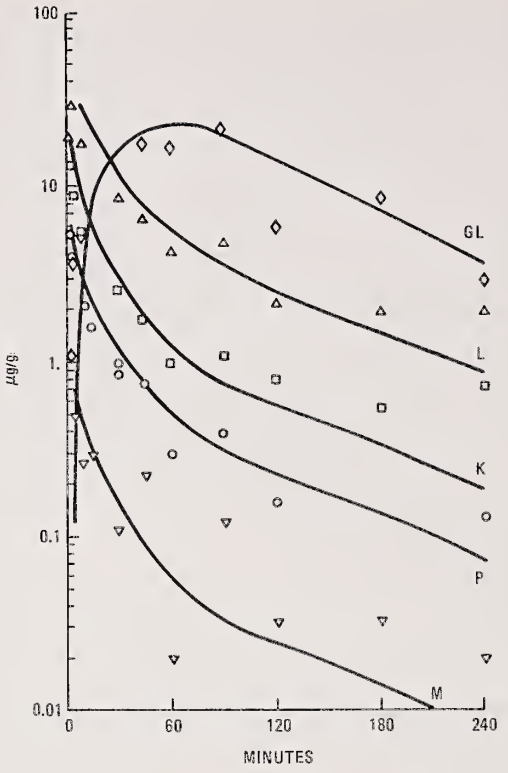
Tissue	$c \times t$ at 48 hr (μg/g × hr)			
	Daunorubicin	Metabolite	Total (daunorubicin + metabolite)	Adriamycin equivalents
Liver	38	87	125	199
Kidney	108	174	282	404
Heart	49	84	133	289
Small intestine	84	100	184	299



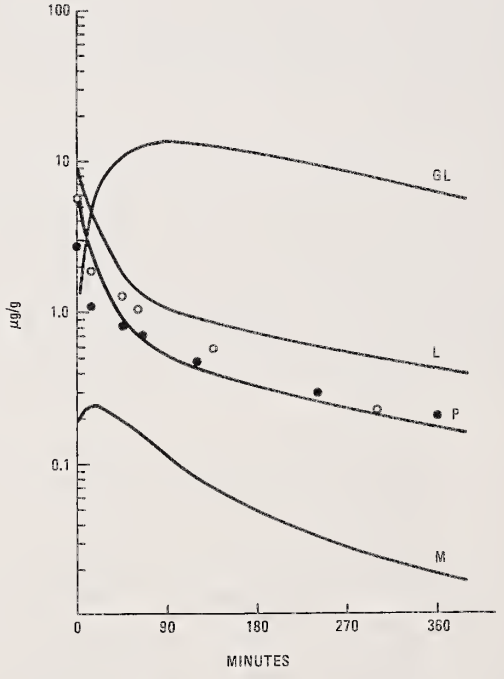
TEXT-FIGURE 2.—Tissues important in methotrexate distribution. Q=blood flow rates; k=rate constants for elimination or absorption; D=delay times present before initial appearance of drug. Subscripts: P=plasma; G=small intestine; L=liver; K=kidney; M=muscle; F=feces; O=Michaelis-Menton process.

back to the animal model scaled appropriately to man. This approach assumes, of course, that a sensitive and specific method is available for the determination of a drug and its metabolites in plasma and other body fluids. It may be necessary to adjust the dose and schedule of the drug in man to achieve the plasma level associated with the particular pharmacologic effect in animals.

Individual differences in drug response may be detected early in patients during phase I trials by observation of variations in drug plasma concentration data or from excretion values. Some individuals excrete drugs so rapidly that adequate plasma levels cannot be obtained. Others excrete so slowly that the resulting high levels of plasma drug will produce adverse effects. Differences in rates of drug metabolism must also be considered because of such factors as the presence of other drugs, variations in tissue enzyme levels, environment, disease state, and genetic differences. For the actual protocol followed in clinical pharmacologic studies, it is obviously similar to the protocol followed in preclinical studies, reviewed earlier in this paper.



TEXT-FIGURE 3.—Model prediction vs. experimental results in mice. GL=lumen of small intestine; L=liver; K=kidney; P=plasma; and M=muscle.



TEXT-FIGURE 4.—Model prediction vs. experimental results in man. GL=lumen of small intestine; L=liver; P=plasma; and M=muscle.

The major tasks of the pharmacologist, well defined in the drug development program, are to devise new analytic methodology and to establish techniques for collecting and interpreting data on the absorption, binding, excretion, distribution, metabolism, and relation of drug-dosage schedule to effectiveness and toxicity. He must further interpret and correlate effects of drugs in animals used for experimental therapy and toxicology and predict the effects of the drug in man, which requires the recognition of quantitative differences as well as qualitative differences of drug disposition between the laboratory animal and man and the establishment of common pharmacologic denominators for relating these differences. This approach is the only logical one for advising the physician on the best way to use drugs in man.

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Experimental Models and Their Clinical Correlations

Stephen K. Carter [1] *and* **Abraham Goldin** [2]

A cancer chemotherapy drug development effort depends on equivalent contributions by chemists, experimental chemotherapists and pharmacologists, and research clinicians, who must collaborate closely to provide large numbers of potentially useful materials, evaluate them in meaningful preclinical systems, select the most promising agents, and evaluate these against the target diseases in man.

The two major approaches in selecting new drugs for preclinical evaluation, with an ultimate view to clinical trial, are 1) synthesis of congeners of known active chemicals with structure–activity relationship data and fundamental biochemical knowledge as guides, and 2) broad screening of heterogeneous classes of chemicals, uncharacterized antibiotics, and plant products to obtain completely new classes of active drugs having mechanisms of action different from the known tumor agents.

The purpose of a screening system is to eliminate the bulk of the negative materials and concentrate for further study those materials exhibiting the greatest promise. The system cannot be designed to detect every compound that will have clinical activity without almost surely including an untenable number of false positive compounds; conversely, it cannot be designed to eliminate every inactive compound without losing some that are active.

For many years, leukemia L1210 (*1*) has been the major screening tool of the National Cancer Institute (NCI) Drug Development Program. The leukemia P388 system is also used for natural products because it has a greater sensitivity for crude fractions. Recently, the program added secondary testing of all L1210- and P388-active materials in antitumor systems, which, we anticipate, will have a greater predictive capacity for drugs active against solid tumors. Initially, these systems will be the B16 melanoma and Lewis lung carcinoma.

CLINICAL RELEVANCE OF THE DRUG DEVELOPMENT PROGRAM

Once it is demonstrated that the model systems for screening and quantitative evaluation can be created, usually taking into account various aspects of the host–tumor–drug interrelationship, a question of their clinical relevance arises. Do the selected model systems identify compounds with a high likelihood of clinical activity? Obviously, if the predictive value of the system is nil, the screening evaluation effort becomes irrelevant to the clinic. In developing a mass screening program, there is also the practical problem of designing a system that, besides being predictive, provides reproducible results and is economically feasible. We believe that leukemia L1210 meets these specifications better than any other system in the program experience.

In leukemia L1210, there is an approximate 2-day lag phase associated with transplantation. This period is followed by a period of log-phase proliferation (generation time, 0.55 day) continuing until host death, at which time the host's leukemia cell population is approximately 1 billion. Screening in L1210 begins on day 2 and is done under conditions far different from the usual clinical situation in acute leukemia, i.e., in vivo treatment of animals bearing relatively small numbers of leukemia cells. Still the worth of a screen must be determined in the end by the clinical results obtained.

Any type of mass screening involves compromise, and chemotherapy screening is no exception. It must be accepted that, of the materials that get through any screen, a certain percentage will be inactive clinically and, unfortunately, some active materials will be missed. The best screen is obviously one that misses the fewest active materials and selects the fewest inactive ones.

Any screen offers four possible results as outlined in table 1. To evaluate a screen fully, the data on all four possibilities should be appraised, but this can rarely be achieved because it is not feasible to test clinically all negative materials to determine the numbers of true and false negatives. Therefore, the chemotherapy screen can only be judged by the positive drugs and the outcome of their clinical use.

The determination of clinical activity and its correlation with findings in the experimental screen depend significantly on an accepted definition of an adequate trial. Drugs selected by a highly quantitative system such as leukemia L1210 may be classified tentatively as false positives if, because of poor absorption, systemic inactivation, or limiting toxicity in the modality of therapy employed, etc., they have not received an adequate trial. As a minimum, an adequate trial requires that a drug be given in the range of the maximum tolerated dose and on an appropriate schedule of administration.

The Division of Cancer Treatment (DCT) conducts the clinical evaluation of a new agent in three phases (table 2) that are specific modifications of those required by the Food and Drug Administration (*2*) and which will be discussed in greater detail in later sections of this paper.

Phase II trials consist of a survey for clinical activity against a panel of 10 signal tumor types. This survey is designed to test thoroughly for drug activity against tumors that are kinetically described as "fast growing" (leukemia, lymphoma) and "slow growing" (colon, breast, lung, etc.). For each signal tumor type, the objective response rate is examined in at least 20–30 patients in controlled crossover designs (*3*). Implicit in

TABLE 1.—*Possible results with any screening system*

Classification	Screen	Clinical trial
True positive	+	+
False positive	+	–
True negative	–	–
False negative	–	+

[1] Formerly, Deputy Director, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014. *Present address*: Director, Northern California Cancer Program, Palo Alto, Calif. 94304.

[2] Chief, Laboratory of Experimental Chemotherapy, Division of Cancer Treatment.

TABLE 2.—*Clinical trials of new drugs developed by NCI*

Phase	Activities
I: Clinical pharmacology	Establish maximum tolerated dose at schedule(s) tested Establish toxicity parameters and determine if toxicity is predictable, treatable, and/or reversible Pharmacologic evaluation Antitumor activity not required
II: Screening for clinical activity	Treat 20–30 patients who can be evaluated with measurable disease in each of a range "signal" tumor types Evaluate on the basis of objective response rate and characteristics of pharmacology, mechanism of action, and cell-cycle sensitivity
III: Trial for recommendations of general use	Controlled clinical trials Combination studies

the use of the 10 signal tumor types is a minimum definition of what constitutes an adequate evaluation of a new antitumor agent. As a minimum, it would be unfair to label a drug "inactive" until it has been adequately evaluated in at least 20–30 patients with most of the signal tumor types and found to have insufficient clinical activity in all of them.

In line with this reasoning, the Cancer Therapy Evaluation Program (CTEP) has instituted an intensive review of all cancer chemotherapeutic agents to see how many have been adequately evaluated by these standards and to provide an historical background for the data rapidly developing on new investigational agents. Table 3 is an outline of the findings on the clinical antitumor activity of the commonly used commercially available agents in seven signal tumor types. The data are given in percent objective response rates; the actual number of responses per total number of patients are in parentheses. It is clear that most of these drugs have not been fully tested against all 7 tumors, and that the practice of listing false positives for any particular preclinical screen for antitumor agents is tenuous.

One cannot determine from such data whether an adequate dosage schedule was used. An optimal therapeutic schedule reflects a variety of factors. In addition to relative sensitivity of a given neoplastic cell as opposed to the normal cells of the body, there is the problem of the optimal body fluid concentration of drug with time. One cannot speak confidently about the relative sensitivity to drugs of different tumors in various hosts without having quantitative data on the blood concentrations of drug with time achieved in the comparative therapeutic trials and the degree of advancement and proliferative states of the respective tumor cell populations. Whereas this information can be obtained readily in experimental systems, such as in animals with leukemia L1210, it is rarely, if ever, acquired in clinical trials.

One of the major factors in the steady improvement in the control of rapidly growing cancer has been the development of more effective therapeutic schedules than were conceived in the early days of cancer chemotherapy. It has been shown repeatedly that optimal schedules can make the difference between a good and a poor response or even success and failure. As Skipper et al. (*4*) pointed out, this can only mean that optimal regulation of the drug concentration (with time) to which both neoplastic and normal cell populations are exposed significantly affects "selectivity." Also, it is clear that optimal schedules for different types of chemotherapeutic agents vary, depending on their mechanisms of action.

The Chemotherapy Program has a protocol for initial schedule and oral route dependency studies against L1210. This protocol, outlined in table 4, was detailed in an earlier screen (*5*). One example of such a study is that for the new drug guanazole [text-fig. 1; (*5*)], which acts as an inhibitor of the enzyme ribonucleotide reductase and shows marked schedule dependency with distinct superiority in results observed when it is given eight times a day on days 1, 5, and 9. This type of schedule dependency is classical for agents that specifically inhibit cells in DNA synthesis. The structures of four drugs, including guanazole, that show this type of dependency are given in text-figure 2. Cytosine arabinoside works via inhibition of DNA polymerase (*6*), whereas the other three all inhibit ribonucleotide reductase (*7–9*). The data for 5-hydroxypicolinaldehyde thiosemicarbazone (5-HP; NSC-107392) in table 5 again show the superiority of the around-the-clock intermittent schedule.

As stated earlier, Skipper et al. (*4*) have indicated that the schedule dependency of drugs may rely in part on their mechanism of action. Table 6, taken from this work (*4*), lists some major drugs categorized by their mechanisms of action versus their kinetic and therapeutic implications. These are separated into four categories: 1) S phase-specific drugs; 2) S phase agents with self-limitation, including the classic antimetabolites such as methotrexate, 6-mercaptopurine, and 5-fluorouracil (5-FU), 3) cell-phase-nonspecific agents with selectivity for populations with high growth fractions, including drugs such as cyclophosphamide, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU); and 4) cell-cycle-nonspecific drugs such as daunorubicin and dactinomycin.

Table 7 (*4*) outlines the schedule dependency in the early L1210 system for these agents, based on the ability to "cure" animals bearing about 10^6 L1210 cells. The S phase-specific agents show the schedule dependency outlined previously. The cell-cycle-nonspecific drugs with selectivity for high growth fraction populations have optimal efficacy when given as a large single dose. The other 2 groups are uniformly unable to cure L1210 on any schedule. Table 8 depicts the schedule dependency based on median percent increase in life-span (% ILS) for the highest nonlethal dose. Here the data for the agents that are S phase with self-limitation hint at superiority for an intermittent schedule but not for one involving around-the-clock therapy.

Methotrexate represents one of the most fascinating examples of the importance of schedule dependency, and its activity in leukemia L1210 shows this dependency characteristic is noteworthy. This effect was first noted by Goldin et al. (*10*), who demonstrated that administration of methotrexate every 4 days was superior to daily injections in two situations: against early L1210 (1 or 2 days after inoculation) and against advanced L1210 leukemia, after other agents had been given to reduce markedly the leukemia cell load. On the other hand, these investigators found that daily administration of the drug resulted in optimal therapeutic effect against advanced disease (in which treatment was not started until 7 or 8 days after inoculation, at a time when the mice had an enormous leukemia cell load). Selawry et al. (*11, 12*) have amply demonstrated that intermittent (twice weekly) is superior to daily methotrexate for remission maintenance in childhood acute lymphatic leukemia.

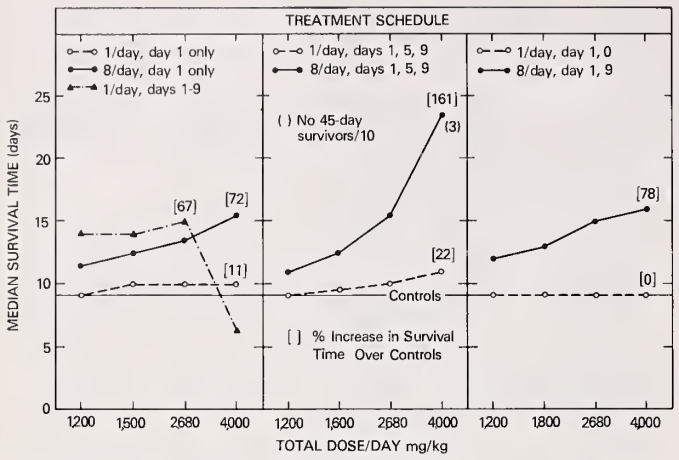
This drug has been used clinically on a variety of schedules.

TABLE 3.—*Clinical antitumor activity in 7 signal tumor types given in percent*[a, b]

Agents	Acute lymphatic leukemia induction	Acute myelocytic leukemia induction	Lymphoma		Adeno-carcinoma of colon	Adeno-carcinoma of breast	Broncho-genic carcinoma	Malignant melanoma
			Hodgkin's disease	LSA and RCS				
I. Alkylating								
Cyclophosphamide	29 (101/347) CR = 13%	10 (8/76) CR = 2.6%	54 (245/452)	LSA 65 (173/276) RCS 56 (122/219)	16 (7/43)	32 (60/189)	33 (168/509)	26 (8/31)
Nitrogen mustard (mechlorethamine)	Acute leukemia 7 (2/29) All prior to 1950		63 (432/682)	LSA 49 (75/154) RCS 18 (3/17)	12 (6/50)	34 (33/98)	21 (81/391)	9 (3/34)
Chlorambucil	NE	16.7 (3/18)	61 (172/282)	LSA 67 (61/90) RCS 29 (18/46)	9 (5/55)	20 (11/54)	8 (2/23)	9 (2/22)
Melphalan (L-phenylalanine mustard	0 (0/27)	NE	32 (22/70)		15 (12/82)	23 (20/86)	10.5 (4/38)	12 (8/66)
Busulfan (myeleran)	NE	3 (1/32)	25 (6/44)		10 (1/10)	0 (0/8)	32 (6/19)	10 (1/10)
II. Antimetabolites								
Methotrexate	40 (63/159)	14 (31/210)	30 (13/44)		17 (19/111)	34 (120/356)	33 (56/167)	9 (2/26)
5-Fluorouracil	5 (1/21) All old studies		26 (6/23)		24 (305/1,270)	28 (323/1,152)	7.5 (12/158)	2.5 (1/42)
6-Mercaptopurine	66 (393/596) CR = 38%	28 (144/521) CR = 11.5%	37 (13/35)		5 (4/75)	13 (6/45)	4 (4/95)	7 (2/30)
Cytosine arabinoside	24 (41/168) CR = 8%	27 (101/502) CR = 20%	29 (12/42)		10 (10/103)	9 (6/65)	0 (0/60)	2 (1/51)
III. Vinca alkaloids								
Vincristine	58 (191/327) CR = 44%	51 (21/41) CR = 24%	56 (74/132)	53 (109/204)	0 (0/26)	21 (47/226)	9 (4/43)	12 (6/53)
Vinblastine	9 (5/63)	26 (25/98) CR = 4%	65 (259/380)	27 (24/89)	7 (5/71)	20 (19/95)	11 (12/109)	15 (8/54)
IV. Antibiotics								
Dactinomycin	NE	NE	50 (9/18)		15 (7/48)	11 (5/45)	0 (0/16)	33 (19/58)
Mithramycin	NE	NE	40 (4/10)		16 (4/25)	16 (5/32)	0 (0/13)	15 (2/13)
Daunorubicin	27 (113/388) CR = 20%	37 (175/469) CR = 28%	18 (11/61)		0 (0/2)	0 (0/2)	0 (0/5)	0 (0/6)
Mitomycin C	NE	NE	47 (16/34)		20 (28/140)	37 (41/110)	21 (29/135)	18 (9/50)
V. Miscellaneous								
Hydroxyurea	12 (2/17)	16 (17/108)	7 (1/14)		7 (4/59)	19 (4/21)	11 (4/36)	22 (47/213)
Procarbazine (matulane)	0 (0/6)	20 (1/5)	70 (212/305) CR = 37%	40 (29/73)	20 (1/5)	5 (1/21)	15 (26/172)	18 (6/32)
Imidazole carboxamide (DTIC)	NE	NE	40 (4/10)		11 (8/71)	10 (4/41)	10 (8/81)	20 (81/419)
BCNU	NE	NE	51 (51/151)	19 (8/43)	13 (17/128)	35 (15/43)	9 (4/46)	20 (17/84)
Methylglyoxal bis guanylhydrazone (methyl-GAG)	7 (1/15)	25 (75/297) CR = 15%	60 (12/20)		0 (0/9)	16 (1/6)	12 (1/8)	NE

[a] Numerator and denominator figures in (*13*).
[b] Abbreviations: LSA = lymphosarcoma; RCS = reticulum cell sarcoma; NE = not evaluated; CR = complete remission.

The pooled reported results in the literature for methotrexate against 6 signal tumors in the Chemotherapy Program classified according to dosage schedule and data for 2 other tumors, malignant melanoma and inoperable head and neck neoplasms, are given in table 9 (*13*). The data reveal that methotrexate has not received an adequate trial in any of these eight major clinical tumor types on each of the five most commonly used dosage schedules. When one further considers the methotrexate schedules in which calcium leucovorin "rescue" was used, the complexity of the situation becomes readily evident.



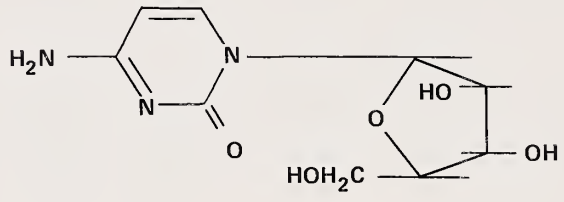
TEXT-FIGURE 1.—Influence of treatment schedule on guanazole activity against ip inoculated L1210.

Clearly, it would be a mistake to call methotrexate "inactive" in cancers of the breast, colon, or lung based on the available data. One could even advance the argument that on selected schedules, this drug may be as active as any single agent officially recommended for these diseases, although obviously large numbers and controlled comparative trials would be required before a definitive statement could be made. However, this situation again illustrates how hazardous it may be to label drugs as false positives for a given screening system.

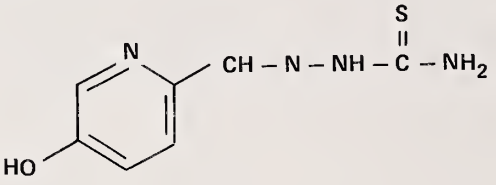
TABLE 4.—*Protocol for initial schedule and route dependency studies against L1210 (ip)* [a, b]

Route	Treatment days	Number on each day	Total number of treatments
ip	1-5	1	5
ip	1-9	1	9
ip	1	1	1
ip	1	8	8
ip	1, 5, 9	1	3
ip	1, 5, 9	8	24
ip	1, 9	1	2
ip	1, 9	8	16
sc and oral	1-9	1	9
sc and oral	1	1	1

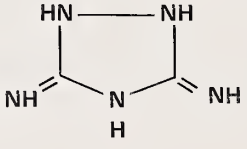
[a] *See* (5).
[b] Experiments conducted in parallel groups of leukemic and normal mice.



NSC - 63,878
Cytosine arabinoside

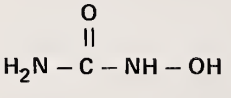


NSC - 107,392
Picolinaldehyde, 5 hydroxy thiosemicarbazone



NSC – 1895
Guanazole

1,2,4-Triazolidene, 3,5-dimino



NSC - 32,065
Hydroxyurea

TEXT-FIGURE 2.—Structures of cytosine arabinoside, 5-HP, guanazole, and hydroxyurea.

TABLE 5.—*5-Hydroxypicolinaldehyde thiosemicarbazone (NSC-107392) vs. L1210* [a]

Treatment schedule, ip	Expts 08-LE-3409, 3502 [b]				Expt 08-LE-3853 [c]			
	Optimum dose, mg/kg [d]	ILS, %	Survivors/total [e]		Optimum dose, mg/kg [d]	ILS, %	Survivors/total [e]	
			L	N			L	N
Once, day 1	2,400	106	1/10	7/8	(600)	Neg [f]	0/10	8/8
Every 3 hr; day 1	150/8	128	4/20	16/16	300/8	78	0/10	8/8
Every day; days 1, 9	1,200	90	0/10	8/8	(600)	Neg [f]	0/10	8/8
Every 3 hr; days 1, 9	1,200/8	175	0/10	8/8	150/8	230	2/10	8/8
Every day; days 1, 5, 9	800	100	0/10	8/8	400	35	0/10	8/8
Every 3 hr; days 1, 5, 9	400/8	240	10/20	13/16	200/8	450	7/10	8/8
Every day; days 1-9	150	104	0/10	8/8	75	88	0/10	6/8

[a] *See (5).*
[b] Suspension in saline plus Tween 80.
[c] Solution in saline plus base (pH 9-10).
[d] Total dose per treatment day. Highest nonlethal dose *in parentheses.*
[e] L = leukemic mice; N = normal mice.
[f] ILS less than 25%.

TABLE 6.—*Mechanisms of action versus kinetic and therapeutic implications* [a]

Agent	Suggested sites of action	Possible end-product inhibition or inactivation	Implication regarding cycle-phase specificity
1. Ara-C	DNA polymerase	DNA	S phase specific
2. Hydroxyurea	Nucleotide reductase	"	"
3. Guanazole	"	"	"
4. 5-Hydroxypicolinaldehyde thiosemicarbazone	"	"	"
5. Methotrexate	Folic reductase	DNA, RNA, protein	S phase with self-limitation
6. 6-Mercaptopurine	Phosphoribosylamine (purines)	DNA, RNA	"
7. 5-Fluorouracil	Thymidylate synthetase: incorporated into RNA	DNA, RNA	"
8. Cyclophosphamide	Reacts with DNA (and other cell constituents)	DNA (others?)	Cycle phase nonspecific but with selectivity for populations with a high growth factor
9. BCNU	?	?	"
10. CCNU	?	?	"
11. Daunorubicin	Complexes with DNA	DNA (others?)	Cycle phase nonspecific
12. Dactinomycin	"	DNA (others?)	"

[a] *See (4).*

DEVELOPMENT OF EXPERIMENT SYSTEMS COMPATIBLE WITH CLINICAL NEEDS

Currently, the DCT is evolving a dual approach to combined therapy in solid tumors (text-fig. 3), which will be discussed in greater detail in a later section. In disseminated (advanced) disease, the activity of single agents is determined and, from these, optimum combination chemotherapy regimens are designed. The active single agents or combinations are then joined with surgery and/or radiotherapy in a combined modality approach to initial treatment of local (early) disease. This dual approach includes four points at which a model experimental system could be useful; three of them involve combination therapy.

The principal consideration in the development of an experimental model is how it is to be put to clinical use; for example:

1) To predict a high degree of activity by unknown compounds or combinations (screening)
2) To predict the success of refinements of therapy with existing compounds or combinations
   a) Elucidate principles behind successful clinical therapy
   b) Predict successful combinations among the myriad of possibilities drawn from active agents and modalities

Among the several possibilities, probably the most feasible and worthwhile objective within present resources would be a system that could predict the success of combinations and modalities. Of course, the validity of the system must be well established before undertaking large-scale assaying. Also, testing of every combination would be practically impossible, and selectivity is required.

With these points in mind, the first critical question to be answered is whether the experimental systems frequently used by DCT will be helpful in predicting active drug combinations. Certainly, a massive source of data already exists from studies in the leukemia L1210 system, the experimental tumor so valuable in detecting active new single agents. Assuming that the L1210 system will be a valid model, we can attack the prob-

TABLE 7.—*Schedule dependency in the L1210 system (early disease before "crowding")* [a]

Agent	Possible end-product inhibition or inactivation	Cures in animals bearing about 10^6 L1210 cells, highest nonlethal dose for each schedule (0.75–1.0 LD10)				
		Single dose: day 2	Every 3 hr (×8); days 2, 6, 10, and 14	Every day; 2–16 days	Every 2 days (×8); 2–16 days	Every 4 days (×4); days 2, 6, 10, and 14
1. Ara-C	DNA (only)	0/10	114/229	0/20	0/10	0/10
2. Hydroxyurea	"	0/20	12/30	0/30	0/10	0/10
3. Guanazole	"	0/10	7/20	0/10	0/10	0/10
4. 5-Hydroxypicolinaldehyde thiosemicarbazone	"	0/10	14/30 [b]	0/20	—	0/20
5. Methotrexate	DNA, RNA, protein	0/60	0/40	0/120	0/30	0/80
6. 6-Mercaptopurine	DNA, RNA	0/20	0/20	0/80	0/10	0/10
7. 5-Fluorouracil	DNA, RNA	0/10	0/30	0/20	0/20	0/10
8. Cyclophosphamide	DNA (and others?)	44/99	0/10	0/40	0/10	0/10
9. BCNU	?	35/50	12/30 (10^7)	1/20	5/10	6/10
10. CCNU	?	357/425	6/60 (10^7)	0/10	5/10	18/40 (10^7)
11. Daunorubicin	DNA (and others?)	0/10	0/30	0/10	0/10	0/10
12. Dactinomycin	"	0/10	0/20	0/20	0/10	0/20

[a] Our goal is to kill leukemia cells faster than they are being replaced for long enough to reduce the viable number to zero, without ever overdosing the host. Clearly (in this model before the leukemia cell population becomes "crowded") optimal scheduling can make the difference between success and failure. Also, it appears that the approach to optimal scheduling is quite different for agents that are: 1) cycle stage specific (e.g., S phase specific); 2) cycle stage specific but self-limiting; 3) cycle stage nonspecific. *See* (*4*).

[b] Three courses rather than four.

TABLE 8.—*Schedule dependency in the L1210 system* [a]

Agent	Possible end-product inhibition or inactivation	Median percent ILS for highest nonlethal dose for each schedule, cures excluded in survival time calculations [b]				
		Single dose: day 2	Every 3 hr (×8); days 2, 6, 10, and 14	Every day; 2–16 days	Every 2 days (×8); 2–16 days	Every 4 days (×4); days 2, 6, 10, and 14
1. Ara-C	DNA (only)	55	271+	135	164	164
2. Hydroxyurea	"	11	117+	61	55	11
3. Guanazole	"	11	188+	163	—	33
4. 5-Hydroxypicolinaldehyde thiosemicarbazone	"	50	100+ [c]	100	—	78
5. Methotrexate	DNA, RNA, protein	40	30	105	128	100
6. 6-Mercaptopurine	DNA, RNA	39	67	53	33	44
7. 5-Fluorouracil	DNA, RNA	64	100	100	132	83
8. Cyclophosphamide	DNA (and others?)	180+	40	30	60	320
9. BCNU	?	184+	184+	50	316+	383+
10. CCNU	?	237+	237+	33	203+	294+
11. Daunorubicin	DNA (and others?)	33	25	27	16	33
12. Dactinomycin	"	50	17	30	55	33

[a] *See* (*4*).

[b] + = some cures.

[c] Three courses rather than four.

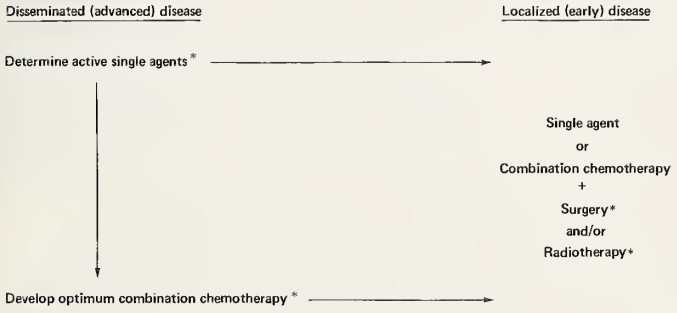
lem of identifying the drug combinations to be tested by either a disease-oriented or a modality-oriented process of selection (table 10).

The disease-oriented process approach may be taken by selection of the 10 (approximate) most active agents used against each of the solid tumor types and hematologic cancers. For example, table 11 lists nine agents for adenocarcinoma of the breast, the best dose schedule for each, and the two-drug combinations (when any two of the nine drugs are used) that have been clinically tested in the disease (*14*). When the most effective agents against each disease are listed, 29 different drugs exhibit established activity when used alone against 1 or more of the 10 tumors shown in table 12. One of the most striking points in these data is how few of the potentially effective two-drug combinations, which could be devised with 9 or 10 most active drugs, have actually been tested in each tumor. Rarely does the number tested exceed 20% of the possibilities, and it is much less in most of the diseases. Even if combina-

TABLE 9.—*Comparison of objective response rates to methotrexate in 8 tumors correlated with schedule used*

Tumor type	Single dose daily, %	Divided dose daily or continuous infusion, %	High single dose intermittent, %	Twice weekly or weekly, %	Loading course monthly
Acute lymphocytic leukemia	35 (51/144) CR = 27	—	80 (12/15)	31[a]	—
Acute myelocytic leukemia	13 (12/92)	25 (10/40) CR = 17.5	—	9 (7/78)	—
Lymphoma	30 (13/44)	—	—	—	—
Breast	41.5 (44/106)	37 (38/102)	—	40 (15/38)	21 (23/110)
Colon	33 (2/6)	23 (8/39)	25 (3/12)	—	9.2 (5/54)
Lung	—	27 (5/18)	25 (5/20)	39 (39/100)	24 (7/29)
Malignant melanoma	0 (0/3)	14 (2/14)	—	—	0 (0/9)
Head and neck	—	33 (13/39)	38 (11/29)	55.8 (43/77)	39 (34/87)

[a] Reported without numbers given; a randomized comparison with daily methotrexate, which gave 28%.



TEXT-FIGURE 3.—Dual approach to combined therapy in solid tumors. Points at which model experimental systems could be used are shown by the *asterisk*.

TABLE 10.—*Possible approaches to selection of drug combinations for experimental studies*

Survey	Approach
Disease oriented	Choose critical tumors Isolate active agents Prospectively test combinations of these agents
Modality oriented	Choose critical drugs or modalities Test these systematically in combination with all other available agents

tions that include drugs with similar mechanisms of action are excluded, which may or may not be a valid step, the gaps are still awesome and may never be filled with the available clinical resources; they emphasize the need and the potential usefulness of testing combinations in a model that has some predictive value.

When the two-drug combinations that have been clinically tested in each disease are reviewed, there are 38 combinations for solid tumors (table 13) and 25 for hematologic cancers (table 14). For convenience, these 63 combinations are indicated on the quick-reference chart of text-figure 4. Unfortunately, few of these combinations have been directly compared with their single components in a prospective controlled fashion.

In considering the evaluation of a new model system, one finds that the only compounds available for testing in it are those with activity that was predicted by older systems. The fact that all these active compounds were not predicted by one system provides a heterogeneity that can be used to advantage in evaluating the new antitumor model. For example, table 15 illustrates this approach in testing the new system against a number of drugs found active and an equal number inactive in L1210 but active in other predictive models. The new system would be highly desirable if it showed activity for all 10 of the L1210-inactive materials and lack of activity for the 10 L1210-active compounds. However, the exact opposite result would be most unattractive. Most systems would lie somewhere between the two extremes, but such an evaluation would be

TABLE 11.—*Single agents active against breast cancer and clinically tested two-drug combinations involving these agents*

Drug	Schedule	Clinical two-drug combinations
Adriamycin	Single dose every 3 wk	Adriamycin + vincristine
Cyclophosphamide	Various, all apparently equivalent	Cyclophosphamide + 5-FU; cyclosphamide + prednisone
5-FU	Daily × 5, or weekly	5-FU + vincristine; 5-FU + vinblastine; 5-FU + prednisone; 5-FU + melphalan
Methotrexate	Daily, daily × 5, or every 6 hr × 24	Methotrexate + thio-TEPA
Vincristine	Weekly	See above combinations
Thio-TEPA	Daily × 2–4	
Vinblastine	Weekly	
Prednisone	Daily	
Melphalan	Daily or daily × 4 every 3–4 wk	

TABLE 12.—*Single drugs most active against the major tumor types and disease-oriented comparison of potential and clinically tested two-drug combinations* [a]

Tumor types	Most single active drugs	Disease-oriented view of two-drug combinations using the most active drugs			
		Number of active drugs	Potential combinations	Combinations actually tested	
				Number	Percent
Solid tumors					
Lung	Cyclophosphamide, mechlorethamine, methotrexate, adriamycin, CCNU, methyl-CCNU, BCNU, procarbazine, hexamethylmelamine, hydroxyurea	10	45	8	18
Breast	Adriamycin, cyclophosphamide, 5-FU, methotrexate, vincristine, thio-TEPA, vinblastine, prednisone, melphalan	9	38	8	22
Gastrointestinal	5-FU, 5-floxuridine, methyl-CCNU, CCNU, BCNU, cyclophosphamide, mitomycin C, methotrexate, adriamycin, streptozotocin	10	45	8	18
Ovary	Melphalan, cyclophosphamide, chlorambucil, 5-FU, methotrexate, dactinomycin, thio-TEPA, hexamethylmelamine, adriamycin	9	36	2	6
Melanoma	DTIC, BCNU, methyl-CCNU, cyclophosphamide, dactinomycin, hydroxyurea, melphalan, vincristine, CCNU, procarbazine	10	45	10	22
Testicular	Dactinomycin, mithramycin, chlorambucil, vinblastine, melphalan, bleomycin, methotrexate, vincristine, mechlorethamine, adriamycin	10	45	7	15
Hematologic malignancies					
Acute lymphocytic leukemia	Vincristine, prednisone, 6-mercaptopurine, methotrexate, daunorubicin, L-asparaginase, cyclophosphamide, cytosine arabinoside, adriamycin, BCNU	10	45	8	18
Acute myelocytic leukemia	Cytosine arabinoside, daunorubicin, adriamycin, 6-thioguanine, 6-mercaptopurine, vincristine, prednisone, cyclophosphamide, BCNU, CCNU	10	45	7	15
Multiple myeloma	Melphalan, cyclophosphamide, prednisone, BCNU, CCNU, vincristine, adriamycin, procarbazine, bleomycin	8	36	8	17
Malignant lymphoma	Cyclophosphamide, mechlorethamine, vinblastine, vincristine, procarbazine, CCNU, BCNU, bleomycin, adriamycin, prednisone	10	45	9	20

[a] *See (17).*

TABLE 13.—*Two-drug combinations clinically evaluated in solid tumors*

Cyclophosphamide + methyl-CCNU [a]	5-FU + streptozotocin
Cyclophosphamide + streptozotocin	5-FU + BCNU [a]
Cyclophosphamide + 5-FU	5-FU + CCNU
Cyclophosphamide + methotrexate [a]	5-FU + methyl-CCNU
Cyclophosphamide + vincristine	5-FU + mitomycin C
Cyclophosphamide + hexamethylmelamine	5-FU + prednisone
Cyclophosphamide + prednisone [a]	5-FU + melphalan
Cyclophosphamide + DTIC	5-FU + vinblastine
Cyclophosphamide + CCNU	5-FU + vincristine
BCNU + mitomycin C	Vincristine + dactinomycin
BCNU + vincristine [a]	Vincristine + DTIC [a]
BCNU + DTIC	Vincristine + adriamycin
CCNU + hydroxyurea	Vincristine + CCNU
CCNU + mechlorethamine	Vincristine + bleomycin [a]
Vinblastine + bleomycin [a]	DTIC + dactinomycin
Vinblastine + melphalan	DTIC + procarbazine
Methotrexate + mechlorethamine	DTIC + methyl-CCNU
Methotrexate + hexamethylmelamine	Dactinomycin + chlorambucil
Methotrexate + thio-TEPA	Dactinomycin + mechlorethamine

[a] Evidence of synergism.

TABLE 14.—*Two-drug combinations clinically evaluated in hematologic cancers*

Prednisone + cyclophosphamide [a]	Cytosine arabinoside + cyclophosphamide
Prednisone + melphalan [a]	Cytosine arabinoside + BCNU
Prednisone + BCNU [a]	Cytosine arabinoside + CCNU
Prednisone + methotrexate [a]	Cytosine arabinoside + 6-mercaptopurine
Prednisone + 6-mercaptopurine [a]	Cytosine arabinoside + 6-thioguanine [a]
Prednisone + cytosine arabinoside	Cytosine arabinoside + daunorubicin [a]
Prednisone + vincristine [a]	6-Mercaptopurine + methotrexate
Prednisone + daunorubicin [a]	6-Mercaptopurine + daunorubicin
Prednisone + adriamycin	BCNU + cyclophosphamide
Prednisone + L-asparaginase	BCNU + procarbazine
Vinblastine + vincristine	BCNU + melphalan
Vinblastine + BCNU	BCNU + bleomycin
	Adriamycin + bleomycin

[a] Evidence of synergism.

TABLE 15.—*Drug activity in L1210 as an approach to evaluating a new screening system*

L1210 Active	L1210 Inactive
Cyclophosphamide	Vincristine
Adriamycin	Bleomycin
Methotrexate	Hexamethylmelamine
5-Fluorouracil	Dactinomycin
Nitrosoureas	Vinblastine
L-Phenylalanine mustard	Streptozotocin
Cytosine arabinoside	Prednisone
6-Mercaptopurine	Dibromomannitol
Imidazole carboxamide	Mithramycin
Hydroxyurea	Myleran
New system →	10 L1210 +
	10 L1210 -

TABLE 16.—*Drug activity in leukemia and solid tumors as an approach to evaluating a new screening system*

"Top 10" leukemia drugs	"Top 10" solid tumor drugs
Vincristine	Cyclophosphamide
Prednisone	Adriamycin
Methotrexate	Methotrexate
6-Mercaptopurine	5-Fluorouracil
Cytosine arabinoside	Nitrosoureas
Daunorubicin	L-Phenylalanine mustard
L-Asparaginase	Hexamethylmelamine
6-Thioguanine	Mitomycin C
Cyclophosphamide	Bleomycin
Hydroxyurea	Dactinomycin
New system →	10 leukemia drugs
	10 solid tumor drugs

helpful in deciding whether to do further testing in a new experimental system.

In another approach, the drugs could be separated on the basis of their activity in hematologic cancers or solid tumors. Table 16 lists the 10 most active drugs for leukemia and solid tumors in the opinion of the CTEP; only two drugs (methotrexate and cyclophosphamide) appear in both lists. If one is interested in uncovering a new experimental system for predicting drugs active in solid tumors, this procedure might be helpful.

TABLE 17.—*Evaluation of B16 melanoma system by correlation of drug activity in L1210, B16, and human solid tumors*

L1210	Drug activity [a]	
	B16	Human solid tumors
L+ or L-	B+ or B-	S++
		S+
		S±
		S-
		SO

[a] L+ and B+ indicate ≥ 50% ILS in system concerned; L-, B- indicate < 50% ILS in system concerned; S++ indicates definite activity in at least 2 major solid tumors in man; S+ indicates clear activity in solid tumors, but less than S++; S± indicates some activity, but not clearly established (includes drugs active in 1 solid tumor but clearly inactive in a wide range of tumors (e.g., DTIC, mithramycin, some hormones); S- indicates clearly inactive in at least 2 solid tumors; SO indicates not adequately tested.

Within DCT, the approaches in tables 15 and 16 have been merged to evaluate the potential of the B16 melanoma system for inclusion in the drug development apparatus. In the analysis, the activity of each drug under consideration is defined in the L1210 and B16 experimental systems and in human solid tumors according to the scheme shown in table 17. The ranking of the drugs in each category of activity in solid tumors is given in table 18.

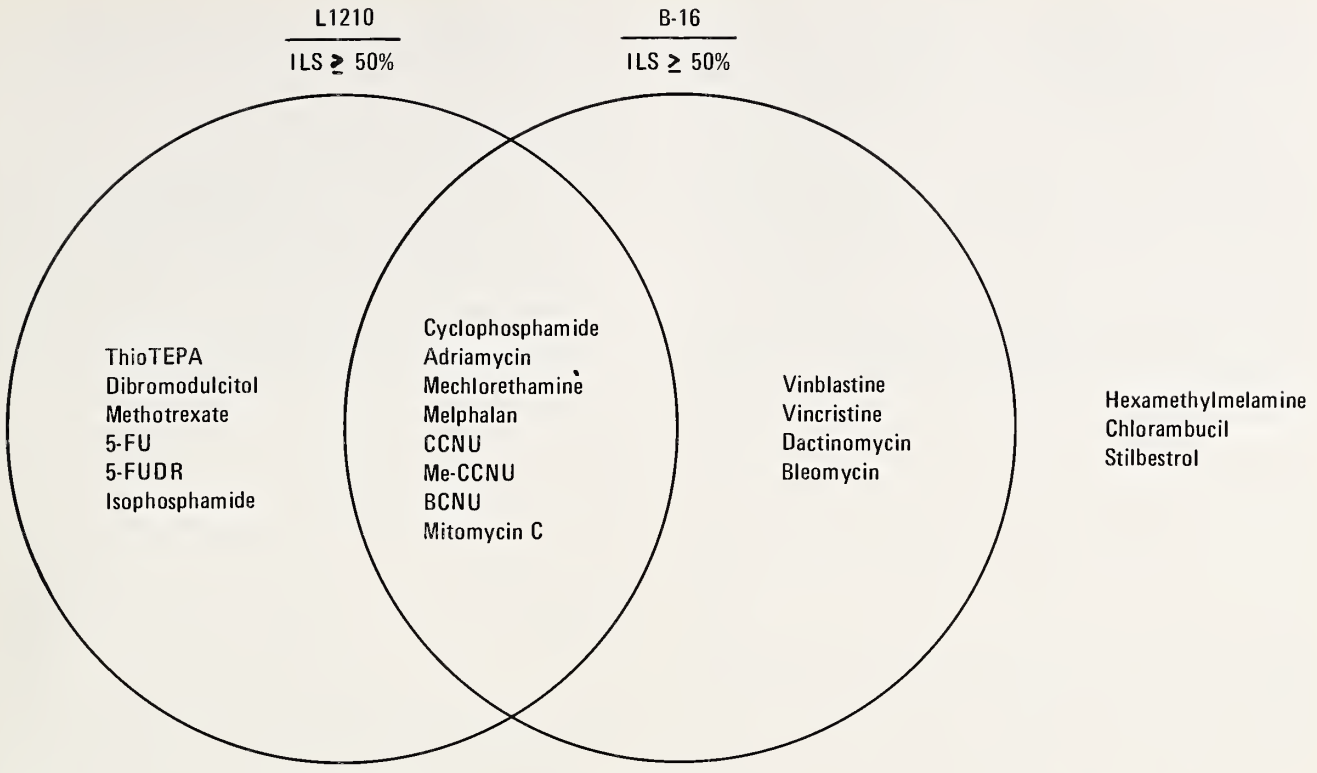
An example of the results obtained in this type of analysis is depicted in text-figure 5. Both experimental systems would have predicted eight of the drugs that are most active in solid tumors, whereas L1210 alone predicted six and B16 four. Both systems failed to predict the activity of three drugs (chlorambucil, hexamethylmelamine, and stilbestrol).

The future of cancer therapy lies in the greater application of combined therapy, which can mean either "combination chemotherapy" using two or more drugs, or it may involve "combined modality therapy" in which single-drug or combination chemotherapy is used as an adjuvant to surgery and/or radiotherapy. Viewing combined therapy strictly in the chemotherapeutic sense, it is quite evident that except for the cures with single drugs in choriocarcinoma and Burkitt's lymphoma, the major successes have been achieved by combination chemotherapy (*15*).

Combinations evaluated in solid tumors ↓ / Combinations evaluated in hematologic malignancies →	Mechlorethamine	Cyclophosphamide	Melphalan	Chlorambucil	ThioTEPA	5-FU	Methotrexate	6-MP	6-Thioguanine	Cytosine Arabinoside	Vincristine	Vinblastine	Mitomycin C	Dactinomycin	Adriamycin	Daunorubicin	Bleomycin	BCNU	CCNU	Methyl-CCNU	Streptozotocin	Hexamethylmelamine	Procarbazine	DTIC	Hydroxyurea	L-asparaginase	Prednisone
Mechlorethamine																											
Cyclophosphamide										(H)								H									(H)
Melphalan																		H									(H)
Chlorambucil																											
ThioTEPA																											
5-FU		S	S																								
Methotrexate	S	(S)			S			H																			(H)
6-MP										H						H											(H)
6-Thioguanine										(H)																	
Cytosine Arabinoside																(H)		H	H								H
Vincristine		S				S						H															(H)
Vinblastine			S			S												H									
Mitomycin C						S																					
Dactinomycin	S			S							S																
Adriamycin											S						H										H
Daunorubicin																											(H)
Bleomycin											(S)	(S)						H									
BCNU						(S)					(S)		S										H				(H)
CCNU	S	S				S					S																
Methyl-CCNU		(S)				S																					
Streptozotocin		S				S																					
Hexamethylmelamine		S					S																				
Procarbazine																											
DTIC		S									(S)			S				S		S			S				
Hydroxyurea																			S								
L-asparaginase																											H
Prednisone		(S)				S																					

TEXT-FIGURE 4.—Quick reference chart of two-drug combinations clinically evaluated in solid tumors and hematologic cancers. S = combination evaluated in one or more solid tumors; encircled S = combination showing evidence of possible synergism; H = combination evaluated in one or more hematologic cancers; encircled H = combination showing evidence of possible synergism.

DCT has developed from the former Chemotherapy Program (*16*) and naturally views combined modality therapy from the standpoint of the chemotherapy involved. Despite the important clinical advances already made with combination chemotherapy, we need to evaluate experimental systems that can provide predictive data of use in the rational design of combination regimens. Rather than attempting after-the-fact validation and understanding of successes in empirical studies, the correct role of the experimental therapist should be to obtain data in anticipation of clinical needs. The clinician and the experimentalist must work closely together if future successes are to come as rapidly as possible.



TEXT-FIGURE 5.—Relationship of the L1210 and B16 systems in predicting drugs most active (S++ and S+) in human solid tumors.

TABLE 18.—*Ranking of drugs by activity in solid tumors*

Activity in solid tumors [a]				
S++	S+	S±	S−	SO
Cyclophosphamide	Vinblastine	DTIC	6-Mercaptopurine	6-Thioguanine
Adriamycin	Vincristine	Mithramycin	Cytosine arabinoside	Daunorubicin
Methotrexate	Chlorambucil	Streptonigrin	Camptothecin	Dibromomannitol
5-FU	Bleomycin	Porfiromycin	TIC-mustard	5-Azacytidine
CCNU	Dactinomycin	Hydroxyurea	L-Asparaginase	Platinum salt
BCNU	Mitomycin C	Procarbazine	TMCA (trimethylcolchicinic acid	ICRF-159
Methyl-CCNU	Dibromodulcitol	Myleran (busulfan)	L-Acetyl-2-picolinoylhydrazine	Yoshi-864
Melphalan	Isophosphamide	TEM (triethylenemelamine)	Tubercidin	Chromomycin A3
Mechlorethamine	Stilbestrol (diethylstilbestrol)	Provera [medroxyprogesterone (17-acetate)]	Cycloleucine	Streptozotocin
Hexamethylmelamine		Testosterone	F3TDR	
5-FUDR		Azotomycin	NSC-17256E	
Thio-TEPA		Prednisone	6-Azauridine	

[a] S++ indicates definite activity in at least 2 major solid tumors in man; S+ indicates clear activity in solid tumors, but less than S++; S± indicates some activity, but not clearly established (includes drugs active in 1 solid tumor but clearly inactive in a wide range of tumors (e.g., DTIC, mithramycin, some hormones); S− indicates clearly inactive in at least 2 solid tumors; SO indicates not clearly tested.

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Phase I Clinical Trials

Stephen K. Carter,[1] Oleg Selawry,[2] *and* Milan Slavik[3]

Phase I studies are limited to clinical pharmacology in patients with solid tumors resistant to all currently available therapy. These patients should have relatively "normal" organ function and must not have received such extensive prior therapy that toxicologic evaluation would be difficult. The estimated survival of patients must be at least 2 months to allow complete evaluation of toxicity. Measurable disease or illness that can be evaluated is not required and a favorable clinical response, although gratifying and significant when it occurs, is not essential at this stage.

The following is an outline of some basic phase I study principles:

1) Initial dose is chosen from large animal toxicology studies.

2) Doses are not escalated in the same patient.

3) Dose escalation is initially rapid with smaller increments of escalation the closer one approaches the toxic range.

4) Three patients are treated at each nontoxic level and 6 at each level showing any toxicity.

5) Antitumor response or lack thereof is not part of the decisionmaking process to move the drug into phase II studies.

Drug doses are given in milligrams per square meter of body surface area because this method achieves better correlation to certain metabolic and excretory functions than does body weight (*1*). Moreover, body surface area can be used as a common denominator for drug dosage in adults and children and in different animal species (*1–4*). Actual determinations of body surface area for various animal species approximate or range within two-thirds of body weight, as shown in text-figure 1, a chart developed by Dr. Oleg Selawry, when he was Chief of the Medical Oncology Branch at NCI. At that time he elucidated many of the phase I study principles discussed here. Enlarged scales for conversion of body weight to body surface area are given in text-figures 2–4 for infants, children, and adults, respectively. The recommended starting dose for initial clinical trial is one-third the minimal toxic dose in milligrams per square meter of body surface area of the most sensitive large animal species, which is usually either rhesus monkey or beagle. It is important to keep in mind that calculations on a milligram per kilogram of body weight basis would not follow this one-third rule.

Three patients are entered at the starting dose level chosen, as described above. At least 1 week should pass between the entry of each of the 3 patients on treatment to decrease the risk of simultaneous toxicity. The patients are kept on the initial dose level for an arbitrary time period, such as 6 weeks, or less time if toxicity occurs.

Subsequent patients are entered at higher dose levels if no dose-limiting toxicity occurs at the preceding dose level. The dose escalations are initially rapid, with smaller increments the closer one approaches the toxic range (modified Fibonacci search scheme), an example of which is given in table 1. This scheme has been used successfully in two phase I studies performed by the Medical Oncology Branch (*5, 6*) and would have been safe for agents of known value, such as methotrexate (*1*).

From 2 to 6 weeks should pass before patients enter the next higher dose level to take advantage of the experience accrued at lower dose levels, especially when delayed and potentially dangerous types of toxicity are expected. Doses should not be escalated in the same patient, because this carries a risk of cumulative toxicity and makes the prediction of a safe starting dose for phase II studies imprecise.

Three patients are used at each nontoxic dose level and 6 at subtoxic levels. More patients can be treated at drug levels with acceptable, reversible toxicity, depending on individual variability of tolerance. Past experience dictates that the tolerated dose will be defined within 5–8 dose steps. Hence between 10 and 30+ patients will be needed for exploration of the initial dose schedule.

It is usually desirable to explore more than one dose schedule of drug administration (e.g., daily vs. widely spaced doses). The starting level for the drug dose on the second schedule is usually 1–2 dose steps below the level of expected toxicity, depending on the experience gained with the initial dose schedule (type of toxicity, reversibility of toxicity, variability of individual tolerance). Exploration of other dose schedules will probably require 9–20 additional patients per schedule.

Toxicity should always be related to a given dose schedule at a given dose level for a given duration of time. Some definitions of toxic doses are in table 2.

TABLE 1.—*Idealized modified Fibonacci search scheme approach to dose escalation in phase I study*

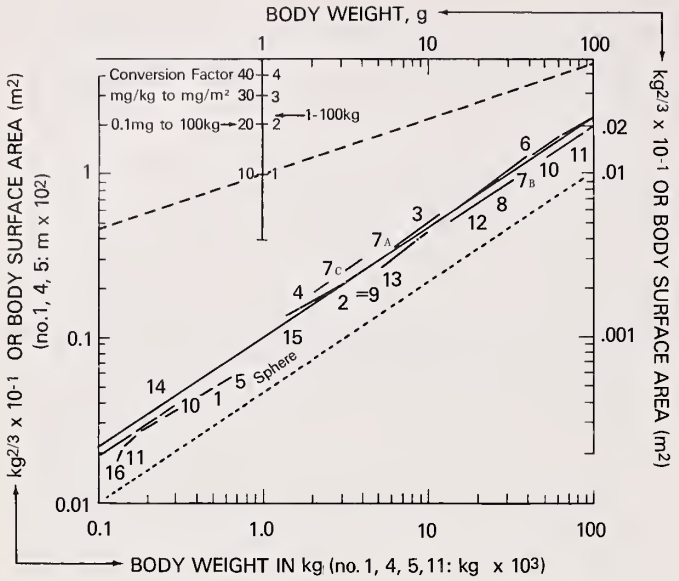
Drug dose	Percentage increase above preceding dose level
n [a]	—
$2.0n$	100
$3.3n$	67
$5.0n$	50
$7.0n$	40
$9.0n$	30–35
$12.0n$	30–35
$16.0n$	30–35

[a] Starting dose = n (milligrams/square meter).

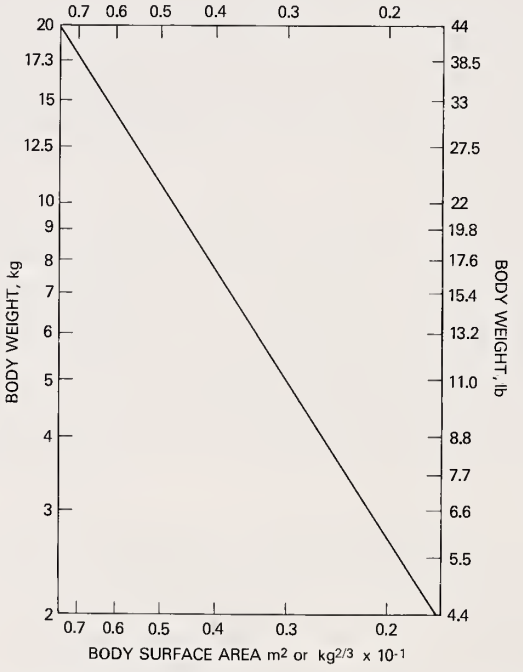
[1] Formerly, Deputy Director, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014. *Present address:* Director, Northern California Cancer Program, Palo Alto, Calif. 94304.

[2] Comprehensive Cancer Center of the State of Florida, University of Miami School of Medicine, Miami, Fla. 33124.

[3] Chief, Investigational Drug Branch, Division of Cancer Treatment.



TEXT-FIGURE 1.—Conversion of body weight to body surface area (m^2) or kilograms × 2/3 for representative animal species and man. Three-log chart, applicable to any body weight. Key: 1=cattle; 2=cat; 3=dog; 4=elephant; 5=horse; 6=man; 7=monkey: A=baboon, B=chimpanzee, C=rhesus; 8=mouse; 9=rabbit; 10=rat; 11=swine; 12=canary; 13=goose; 14=pigeon; 15=lizard; and 16=turtle.



TEXT-FIGURE 2.—Table for conversion of body weight to body surface area (m^2) expressed as kilograms (2/3) × 10^{-1} for infants.

Tumor masses are counted and measured at regular intervals if possible. Measurable tumor masses are defined as the product of the longest times the widest perpendicular diameter. The third dimension can be added when possible. Measurability is arbitrarily defined as reproducibility of simultaneous measurements within 50% by independent observers. Definitions of response are outlined in table 3. It is important to emphasize that antitumor response, or the lack thereof, is not part of the decisionmaking process to move drugs into phase II study.

TABLE 2.—*Toxicity definitions in phase I study*

Term	Definition
Subtoxic dose	A dose that causes *consistent* changes of hematologic or biochemical parameters and might thus herald toxicity at the next higher dose level or with prolonged drug administration (Example: Consistent, drug-related decrease of thrombocytes without dropping below an arbitrarily defined "toxic" level of 100,000 per mm^3)
Minimal toxic dose	The smallest dose at which 1 or more of 3 patients show consistent, readily reversible drug toxicity.
Recommended dose for therapeutic trial	The dose that causes moderate, reversible toxicity in most patients
Maximum tolerated dose	The highest safely tolerable dosage

TABLE 3.—*Definitions of tumor response*

Term	Definition
1) Complete tumor regression	Complete disappearance of all recognizable tumor masses and/or biochemical changes directly related to the tumor
2) Complete remission	Complete tumor regression as above, *and* complete disappearance of all indirect (host-mediated) symptoms, signs, hematologic, and biochemical parameters
3) Partial regression	Greater than or equal to 50% decrease of one or more tumor lesions in the absence of progression or occurrence of new lesions elsewhere
4) Static disease	Changes smaller than those outlined under 3 and 5
5) Progressive disease	Occurrence of any new lesion or increase of any measurable lesion by more than or equal to 50%, irrespective of regressions elsewhere

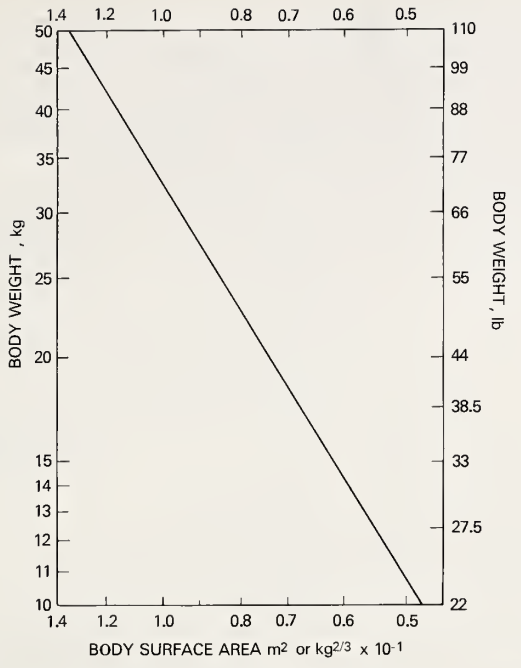
As in any clinical study, the design of a phase I trial must encompass a number of critical variables, such as 1) initial dose, 2) dose schedules, 3) dose escalation procedure, 4) number of patients treated on any dose schedule, 5) data collection, 6) pharmacology, and 7) criteria for moving to phase II studies.

This presentation, rather than directly answering each variable, is intended to stimulate discussion by an examination of the advantages and disadvantages of different approaches used in phase I studies of various drugs.

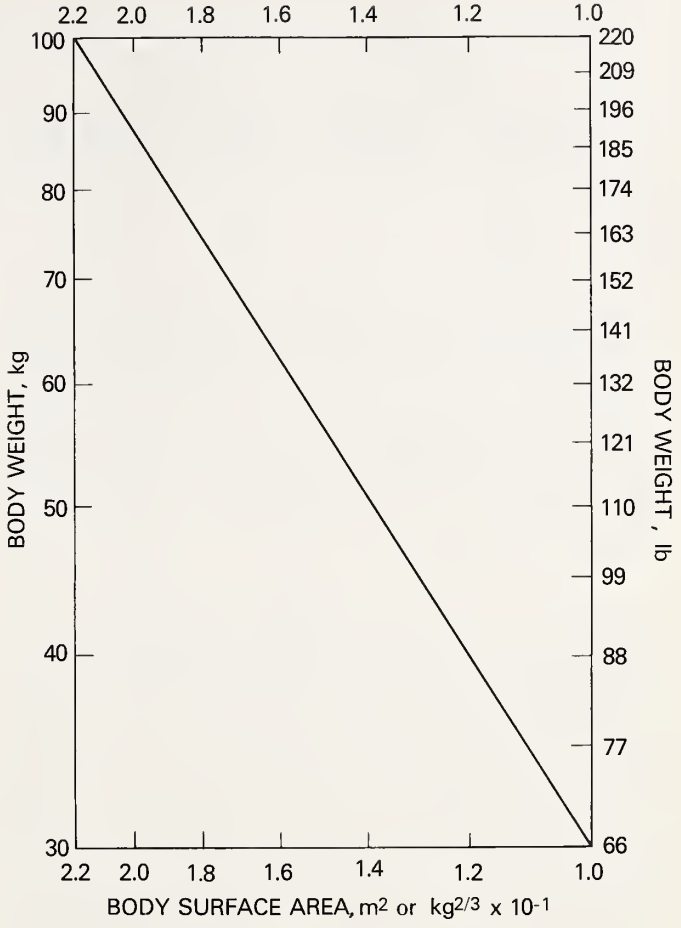
INITIAL DOSE

The initial dose for phase I studies may be chosen on the basis of rodent or large animal data, or clinical data obtained with an analogue or from foreign clinical trials.

Several investigators, particularly in Europe, the Soviet Union, and Japan, have used rodent data to predict an initial dose. For example, a protocol of the Pharmacology Committee of the Soviet Ministry of Health states that one-thirtieth of the maximum tolerated cumulative dose for rats, given over 15



TEXT-FIGURE 3.—Table for conversion of body weight to body surface area (m^2) expressed in kilograms $(2/3) \times 10^{-1}$ for children.



TEXT-FIGURE 4.—Table for conversion of body weight to body surface area (m^2) expressed in kilograms $(2/3) \times 10^{-1}$ for adults.

days in milligrams per kilogram of body weight is used for alkylating agents, whereas lower starting doses are recommended for drugs from other chemical classes. Since all three drugs obtained by NCI from the Soviet Union are alkylating agents, rodent data most probably were the basis for the initial dose in phase I studies.

Most European countries accept the approach of using three animal species, one of them nonrodent, in preclinical toxicity studies. Since the most sensitive species is used for prediction of the initial dose, a rodent species might become the predictive one. Several drugs (e.g., cytembena) have been tested by this approach.

Freireich et al. (*3*) reviewed a large volume of experimental data and showed that there is close relationship between the LD10 (lethal dose for 10% of the treated animals) in rodents and the maximum tolerated dose (MTD) in man when the values are given on a milligrams per square meter basis. However, their conclusions strongly emphasize the dangers of attempting direct extrapolation of animal toxicity data to MTD in man. They did not suggest that it would be wise to convert mouse or rat LD10 to milligrams per square meter and start clinical trials in man at one-third this level. However, calculation of the MTD in these terms has been recommended and is used in Europe (*7*).

Using Freireich's conversion factor for estimation of the human MTD in their phase I study with mycophenolic acid, Knudtzon and Nissen (*8*) found figures ranging from 50 (mouse data) to 17–50 (monkey data) to 2–4 mg/kg (rat data). These authors chose an initial starting dose based on the rat data that predicted the lowest MTD for man (3 mg/kg or 150 mg/m^2). The MTD established in their study ranged between 1,000 and 1,500 mg/m^2 daily, which is about 10 times the MTD originally calculated from the rat LD10 data. This case clearly illustrates the use of rodent data for predicting the initial dose in man.

Large-animal toxicity data have been widely used for prediction of the initial dose in man. These large-animal species include the cat, which is used in the U.S.S.R. and is regarded as particularly predictive for hematologic toxicity and the "mini pig" that is used in European countries. However, the best experience with large-animal predictive models has been achieved with dogs and monkeys in the United States.

The large-animal dose levels that might be selected in the initial phase I dose are 1) highest nontoxic dose (HNTD), 2) toxic dose low (TDL), 3) toxic dose high (TDH), and 4) lethal dose (LD).

Although one-third of the TDL in the most sensitive species, expressed in milligrams per square meter of body surface area, has been the approach for years, the critical questions remain: Is this really the best starting level? Is it safe enough? Can it be improved? The Cancer Therapy Evaluation Program is now conducting statistical analyses of the applicability of animal toxicity data to prediction of drug toxicity in man.

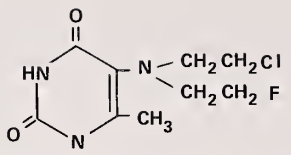
The use of foreign clinical data entails different approaches for determining an initial dose. One may select the full dose or, particularly if there may be population differences in sensitivity to the drug, a fraction of the dose can be used. If the latter choice is made, then the proportion to use must also be determined, and this often depends on the scientific quality of the original investigation.

The NCI program has been using approximately one-fifth of the therapeutic dose used in foreign studies. The initial dose

TABLE 4.—*Chromomycin A3 (NSC-58514) toxicity ranking*[a]

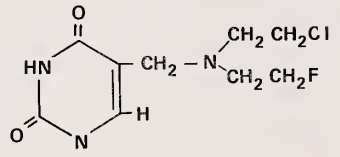
Dose	Dog		Monkey	
	mg/kg/day for 5 days	mg/m²/day for 5 days	mg/kg/day for 5 days	mg/m²/day for 5 days
HNTD[b]	—	—	—	—
TDL[c]	0.025	0.5	0.025	0.3
TDH[d]	0.1	2.0	0.05	0.6
LD[e]	0.15	3.0	0.1	1.3

[a] Dose recommended for initiation of phase I trial: 0.1 mg/m²/day iv.
[b] Highest dose at which no hematologic, chemical, clinical, or pathologic drug-induced alterations occurred; doubling this dose produces these alterations.
[c] Lowest dose to produce drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose produces no lethality.
[d] Dose to produce drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose produces lethality.
[e] Lowest dose to produce drug-induced death in any animals during treatment or observation period.



Fluorodopan (USSR)

5-(2-Chloroethyl-2-fluoroethylamino)-6-methyluracil



(Czechoslovakia)

5-(2-Chloroethyl-2-fluoroethyl)-aminomethyluracil

TEXT-FIGURE 5.—Extremely similar structures of two isomers, dosages of which vary greatly.

of chromomycin A3 recommended for beginning phase I trial (0.1 mg/m²), which was calculated from large-animal data, was about the same as if it had been estimated on the basis of one-fifth of the therapeutic dose (0.7 mg/m²) used in Japan and South Africa (table 4). However, other drugs may not show this type of correspondence. For instance, there is a tremendous difference between one-fifth of the therapeutic dose of 5-azacytidine used in Europe and the initial starting dose estimated in this country on the basis of the large-animal data. If we had started with one-fifth of their therapeutic dose, which was 75 mg/m², we would have avoided underestimation of the initial dose (3 mg/m²) as well as the supply crisis that followed.

The question of use of the clinical data obtained with analogues is also important and interesting, as well as contradictory. Text-figure 5 shows the structure of two analogues, as a matter of fact isomers; one (fluorodopan) was developed in the U.S.S.R. and the other in Czechoslovakia. Although the structures are extremely similar, the difference in doses is large (37 mg/m² dose for fluorodopan vs. 1 mg/m² dose for the isomer). Thus using the dose of the Russian drug as a basis for the dose of the Czechoslovakian analogue would have had fatal consequences. On the other hand, the Czechoslovakian drug was introduced in clinical trial as a fluorinated analogue of ypenyl. The starting dose was based on clinical data available on ypenyl and on the results of comparable preclinical toxicity. Therefore, the same dose was used for the fluorinated analogue and the parent drug. The trial, conducted under double-blind conditions, revealed no difference in therapeutic or toxic effects between the two drugs.

DOSE SCHEDULES

The choice of an initial schedule for phase I study is aided by information from various sources, including schedule-dependency data in rodent tumor systems, data on cell-cycle sensitivity and mechanism of action, pharmacology investigations, and schedules used in studies of large-animal toxicology. Schedules usually followed in NCI-sponsored phase I studies are: 1) single dose, 2) daily × 5 or 10, 3) twice weekly, 4) weekly, and 5) continuous infusion.

These schedules, which generally result from schedule-dependency studies in mice, raise the question of whether the extrapolation to man is relevant without correlation with metabolism and drug disposition. Are these really the optimal dose schedules? Should we proceed in phase I study with only one schedule or with two, three, or all the schedules mentioned? Can we perform better analyses to determine the optimal dose schedule? Are there other schedules that need to be explored? Again, some examples can be given that raise more questions than they answer.

The schedule-dependency data in L1210 leukemia in mice for isophosphamide, an analogue of cyclophosphamide, show clearly the superiority of a single over a daily dose schedule. Results of the phase I study conducted in the NCI–VA Oncology Branch with this schedule are clear and simple with 5 g/m² as the MTD. Unfortunately, the first message from the phase II studies has also been clear and simple. It suggests no superiority of this drug over cyclophosphamide or any confirmation of the clinical data in Europe. Since the European investigators are advocating a daily × 5 schedule as superior for this drug, should we not proceed with the daily schedule?

Quite the opposite example is found for another drug recently used in phase II studies, i.e., 5-azacytidine. Schedule-dependency studies show activity in both daily and intermittent schedules. In table 5, we are facing the consequences. The question might be raised again: Are we wasting our resources or can we do better analyses based on these data?

DOSE ESCALATION PROCEDURE

Dose escalation in phase I studies may either be done in the same patient or be restricted to separate patients. The first procedure is more economical in patient resources but risks cumulative toxicity, whereas the second has the opposite effect.

Also, the techniques of escalation might be different (table 6). Standard increments of 200% of the original dose were used in the phase I study in mycophenolic acid. In their joint effort, England and Scotland finally reached the MTD, which was about 30 times higher than the initial one. Since the modified Fibonacci search scheme for dose escalation has been used and recommended in the Division of Cancer Treatment (DCT), it is detailed in table 1. However, it may totally fail if the initial dose is low, as shown in text-figure 6.

TABLE 5.—*Phase I studies with 5-azacytidine (NSC = 102816)*

Study [a]	Number of patients evaluated	Dose schedule	Dose, mg/m²			Incidence of toxicity, %				Pruritic follicular skin rash
			Start	Highest escalation	Maximum tolerated	Gastro-intestinal	Hemato-logic	Hepato SGOT 40 U	Fever	
McCreadey M. D. Anderson Hospital	39 [b]	Daily × 5, iv every 21 days	2.2	275	225	75	60 [c]	—	Occa-sional	
	12 [d]	Daily × 5, iv every 14 days		400	300				Common	
Karon CCA 020	37 [e]	Daily × 5, iv every 14 days	2.0	200	150–200	Most	+ [c]	—		50
Weiss COG 7110 [f]	30	Daily × 10 iv	0.03/kg (1.11)	2.4/kg (88)	1.6/kg (59.2)	83.3	90 [c]	23.3		6/6
Vogler SEG 137 [f]	24	Twice/wk, iv × 8 wk	50	200	150	87.5 [c]	29.5 [g]	—	—	—
Snider	15	Weekly iv × 4	200	633	500	100 [c]	33.3	—	—	—
	12	Daily × 5, 9 days off, repeat	50	163	150	100 [c] Nausea, vomit-ing, diarrhea	50 [c] Leuko-penia, throm-bocyto-penia	—	—	—

[a] Data on file with Investigational Drug Branch, NCI.
[b] Solid tumors.
[c] Dose-limiting toxicity.
[d] Acute leukemia.
[e] Children with acute myelocytic leukemia and acute lymphocytic leukemia.
[f] Pharmacokinetic study included.
[g] White blood cell count <1,500.

TABLE 6.—*Approaches to dose escalation in phase I study*

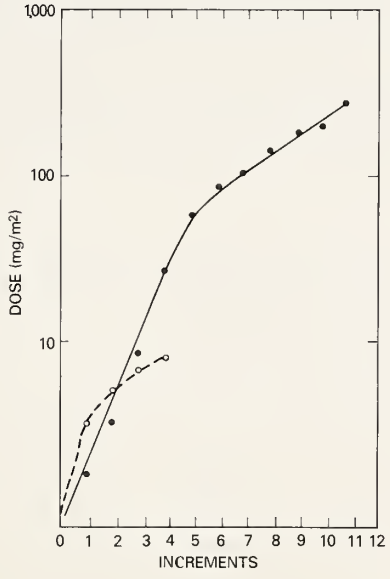
Increment steps	Escalation increments			
Initial dose	100	100	100	100
1	200	200	200	200
2	400	300	350	300
3	800	400	525	450
4	1,600	500	700	675
5	3,200	600	925	1,000
6	6,400	700	1,225	1,500
7	12,800	800	1,625	2,250
8	25,600	900	2,050	3,375
9	51,200	1,000	2,750	5,000

NUMBER OF PATIENTS TREATED

The number of patients who can be treated at any given dose level in a phase I study ranges from 1 to 10 or more. However, economic feasibility and statistical significance are two extreme determinants. In the DCT, at least 3 patients are treated for each dose level.

DATA COLLECTION

In the collection of data, a question might be raised if standard criteria of toxicity and a unified system of data reporting should be used. The personal freedom to report the study in one's own way and procedures offering better statistical analyses, possibly by computer, should be the main determinants to be balanced in this area.



TEXT-FIGURE 6.—Dose escalation steps with 5-azacytidine as compared with idealized escalation with Fibonacci search scheme; ——— = actual escalation; ----- = idealized escalation (from Karon M: Personal communication).

PHARMACOLOGY

In phase I studies a critical question is: Should the pharmacokinetic assays be included in the phase I studies? If they should, at which dose level should they be implemented in the study? Should comparative pharmacology be performed to elucidate the toxicity and/or lack of activity in phase I studies? These are questions that have been posed at many discussion meetings in the United States. It has been generally recommended that 1) preclinical pharmacology should be defined before the drug is given for the first time in man, and 2) pharmacologic studies should be implemented in phase I trials.

Pharmacologic studies in phase I trials can be initiated at the first dose level, at the level showing first toxicity, or at the MTD.

The advantages of starting pharmacology at the initial dose level include early indications of unique pharmacologic situations, early hints of improper choice of schedule, and early comparative pharmacology with other animal species. A disadvantage of starting at the initial dose level is the potential waste of resources in an investigation being done at levels without biologic activity. The critical question is: Should the implementation of the pharmacologic disposition of drugs into phase I studies be obligatory? Should we withhold entering a drug in phase I if the methods for in vivo determination of drug and preclinical pharmacology disposition are not available?

CRITERIA FOR PASSAGE TO PHASE II STUDIES

What is the definition of failure of phase I study? Does it mean only that the drug revealed serious unpredictable and irreversible toxicity in man, which had not been predicted in animal toxicity studies? Or, should lack of activity also be considered? Neither of these questions is easy to answer.

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Phase II Clinical Trials

Stephen K. Carter[1] *and* **Oleg Selawry**[2]

Phase II studies are designed to determine whether a new drug exhibits antitumor activity worthy of further clinical evaluation. The phase II trial, which was not planned to give definitive answers on the ultimate value or role of a given drug (the purpose of the larger phase III studies to be considered in the paper that follows), is a screen for antitumor activity and contains the imperfections of any screening system.

The efficacy of a compound must be examined in as many aspects as possible; it is not just a question of whether a drug is effective, but to what degree it is effective and how it compares with other drugs used to treat a given tumor. In the initial phase II trial, the estimated maximum tolerated dose derived in phase I studies is usually chosen to test the efficacy of the drug. In most cases, trials are conducted with only one dose and one schedule. Unfortunately, it is not possible to be certain that this dose schedule is anywhere near optimum with regard to therapeutic effect. Animal studies make it clear that therapeutic effect of many active agents is highly dependent on the dose schedule and so, ideally, without comparative pharmacology for animals and man, different schedules and doses ought to be used in phase II trials. However, this is rarely feasible.

On completion of phase II clinical studies, it should be possible to make a reasonable judgment regarding the degree of efficacy and the nature of adverse effects at a particular dose schedule. Whether or not further large-scale studies should be performed rests on consideration of those factors, which can be summed up by the concept of the risk-to-benefit ratio. When efficacy is considered the critical point, as Gehan and Schneiderman (*1*) have pointed out, the decision to be reached is whether the agent could be or is unlikely to be effective in $x\%$ of patients or more. An answer can usually be obtained after studying a relatively small number of patients.

One approach to the problem of determining the minimum size of sample for a phase II study is given by Gehan (*2*). Table 1 gives the number of patients necessary for a decision as to whether an agent warrants further study or is not likely to be of a given true therapeutic effectiveness at given levels of rejection error. Rejection error (β) is the chance of failing to send an agent on to further study, when it should have received further consideration. Thus if one is interested in an agent of 25% effectiveness and is willing to accept a 10% rejection error, a sample of 9 patients is necessary. This number was derived by an assumption that the true effectiveness of the agent is 25% and calculating that the chance of 9 consecutive failures is less than 10%. If all 9 patients failed to respond, further study of the agent could be stopped, because with a true response rate of 25%, one or more responses would have been observed with a chance of over 90%.

This approach involves several simplifying assumptions. First, response is not an all-or-none phenomenon and there are degrees thereof. Response must be defined so that when one or more responses have been observed it is meaningful to say the agent is worth further study. The definition of response may not pose serious difficulties in diseases such as solid tumors, in which agents have been largely ineffective up to now. When transient regressions or partial benefits are regularly observed with presently available agents, it may be reasonable to define "response" as a marked objective improvement of some minimum duration in a patient's disease. Second, the number of patients given in table 1 is based on the assumption that the chance of response is the same for each patient; that patients' responses are not homogeneous but can often be subdivided into groups having different probabilities of response is known. Consequently, the numbers given in table 1 should be taken as approximate and as referring to an "average" level of therapeutic effectiveness of interest. Third, some prior knowledge may exist concerning the approximate level of effectiveness of the agent before the phase II study and no allowance was made for this. Finally, when the tumor types are in the phase II trial, the total number of patients needed is almost certainly less than k times the number obtained from table 1 because of the relationship between response in one tumor category and response in another. Since it is not possible to specify the relationship among response rates in various tumor categories, no formal adjustment has been made for this.

Agents with effectiveness of lower order than that of interest may have a substantial chance of being passed to further study by a phase II trial. For example, if one studies 9 patients in

TABLE 1.—*Number of patients required for phase II trials of an agent for given levels of therapeutic effectiveness and rejection error*

Therapeutic effectiveness, %	Number of patients required with a rejection error of:	
	5%	10%
5	59	45
10	29	22
15	19	15
20	14	11
25	11	9
30	9	7
35	7	6
40	6	5
45	6	4
50	5	4

[1] Formerly, Deputy Director, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014. *Present address*: Director, Northern California Cancer Program, Palo Alto, Calif. 94304.

[2] Comprehensive Cancer Center of the State of Florida, University of Miami School of Medicine, Miami, Fla. 33124.

search of an agent of 25% effectiveness (with 10% rejection error), there is more than a 30% chance that one or more responses will be observed, even if the agent has only 5% true therapeutic effectiveness. Such a chance might be acceptable when no agent of any real effectiveness exists for a given disease, but it may not be satisfactory for diseases for which agents of a reasonably high order of effectiveness already exist.

The number of patients for a phase II study can be specified in many different ways. Table 1 presents the *minimum* needed for an idealized phase II study in which response rate is low and qualitative, patients' responses are homogeneous, and there is no correlation among response rates in various tumor categories. The plans are designed to reject ineffective agents quickly and send on to further trial those that have some effectiveness.

When a regimen has passed a phase II trial or was sufficiently effective in a phase I trial, it will nearly always be desirable to study additional patients (follow-up trial) to obtain a more precise estimate of effectiveness. Usually, only few patients are needed in phase II, so more might be studied before starting a phase III trial. Gehan (*2*) provided the number of additional patients to be studied to estimate the true therapeutic effectiveness with a specified (albeit approximate) precision.

The Division of Cancer Treatment (DCT) has decided to use as its screen a panel of 10 tumors known as "signal" tumor types:

Adenocarcinoma of the breast
Adenocarcinoma of the colon
Bronchogenic carcinoma
Adenocarcinoma of the pancreas
Ovarian cancer
Malignant melanoma
Acute myelocytic leukemia
Acute lymphocytic leukemia
Lymphomatous disease
Malignant gliomas

Adenocarcinoma of the breast, colon, pancreas, and ovary, and bronchogenic carcinoma are used to screen against the small growth fraction solid tumors that are traditionally the most resistant to chemotherapy. The acute leukemias and lymphoma have been chosen to represent the rapidly growing and more chemotherapeutic-sensitive tumor types. It is believed that this kind of disease-oriented phase II trial is superior to the often used general phase II trial, in which a wide range of tumor types is treated, but only a few in numbers large enough to give meaningful results. Frequently in the older literature, a drug was described as "inactive," based on a low percentage of responses in 50 or so patients, but only 2 or 3 patients with colon cancer or breast cancer were treated.

The leukemias and lymphomas are, in one sense, the easiest to evaluate from a phase II point of view, as response criteria are well established and readily accessible in almost every patient. In another sense, however, they are difficult to evaluate because the patients reaching the stage of phase II trial have usually been through extensive prior chemotherapy and are at a far advanced and relatively refractory stage of their disease. Given the great potential of single agents and combinations for achieving high complete remission rates, it is particularly difficult to establish the effectiveness of a new drug. That criteria other than simply percent activity must govern whether a drug passes the phase II screen in the rapidly growing tumors is recognized. A drug without bone marrow toxicity that could be easily included in new combination regimens would obviously be superior to a traditional marrow-toxic agent with a similar response rate. Drugs that enter pharmacologic sanctuaries such as the central nervous system are highly desirable, as are drugs with the ability to kill nonproliferating or the so-called G_0 cells.

In the solid tumors the problems have a different emphasis. Here complete remission by a single agent is, unfortunately, a rare occurrence and partial response occurs in the range of 20–30% for most active agents. This means that the sensitivity level for activity can be placed lower, and patients can be treated at an earlier therapeutic stage in relation to drugs. The major problems in this area are the lack of a clear-cut, satisfactory parameter for response and the heterogeneity of the patient population available for study.

SPECIFIC CONSIDERATION IN GASTROINTESTINAL CANCER

Essential to the evaluation of clinical data on a new agent is a knowledge of what standard agents have accomplished, so that rational decisions about further study can be made. In support of this, the DCT Cancer Therapy Evaluation Program (CTEP) has data on over 2,220 cases of large-bowel carcinoma treated with standard commercially available antineoplastic agents (table 2).

Perusal of the data shows that the most active agents are 5-fluorouracil (5-FU; 305/1,270=24%) and mitomycin C (28/140=20%). The alkylating agents appear to have a slight degree of activity with cyclophosphamide leading the list (15/75=20%). Recent data from the Mayo Clinic show an even higher response rate for high-dose, intermittent (30 mg/kg every 3 wk) administration of cyclophosphamide, and the full potential of this agent in gastrointestinal cancer may only now be realized. From the data in table 2, it would appear that any new agent that showed activity in the range of 15–20% in phase II study in large-bowel cancer would be worthy of consideration in phase III evaluation.

In carcinoma of the pancreas and stomach, only 5-FU has been reported in the literature to a degree that makes evaluation meaningful. The response rate to 5-FU is 23% (29/125) and 40% (200/511) in the pancreas and stomach, respectively. Clearly, a great deal of phase II work needs to be done in these tumors.

TABLE 2.—*Cumulative data on the activity of standard chemotherapeutic agents against large-bowel cancer*

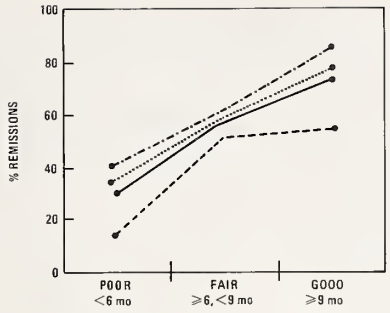
Drug	Number of patients treated	Number of responses	Response, %
Nitrogen mustard	50	6	12
Cyclophosphamide	75	15	20
Chlorambucil	55	5	9
L-Phenylalanine mustard	82	12	15
Busulfan	10	1	10
5-Fluorouracil	1,270	305	24
Methotrexate	111	19	17
6-Mercaptopurine	75	4	5
Arabinosylcytosine	103	10	10
Vincristine	26	0	0
Vinblastine	71	5	7
Dactinomycin	48	7	15
Mithramycin	25	4	16
Mitomycin C	140	28	20
Hydroxyurea	59	4	7
Procarbazine	5	1	20
Dimethyl triazenoimidazole carboxamide	71	8	11

Some of the problems inherent in phase II trials in gastrointestinal cancer can be seen in table 3, which outlines the results of rapid injection of 5-FU for treatment of advanced carcinoma of the large bowel (*3*). It shows a range of objective regression rates of 8–85% with an identical drug schedule used in all studies. The classic explanation for this discrepancy of results is that the parameters of response were different, but much more likely is that patient selection was the key to the problem.

There are three critical variables in any clinical evaluation of therapy: the state of the patient before therapy, the therapy itself, and the state of the patient after therapy. In chemotherapeutic trials in solid tumors, the emphasis is almost exclusively on the last variable, and the pretherapeutic variables are barely considered.

The data of Schneiderman (*4*) are an example of the importance of patient selection and pretherapeutic variables (text-fig. 1). The response to chemotherapy of patients with lymphoma, correlated with their survival, is estimated by the investigator before the onset of therapy. The study was a comparison of three alkylating agents; for each agent, the response rate was dramatically higher for those with an estimated survival of greater than 9 months as compared with those having an estimated survival of less than 6 months. Although it is obvious that drug A is superior to drug C, it is also easy to imagine the circumstance in which patient selection alone could make drug C appear equal to drug A. The implications of such a circumstance for phase II trials in a given tumor with only 20–30 patients are immediately obvious and not a little bit unsettling. What is also impressive about this correlation is that an estimate of survival is made by every good clinician, almost subconsciously, but is rarely written down for inclusion and correlation of clinical results. If this correlation could be supported by additional studies, it would be a simple and powerful tool for increasing the sensitivity of phase II trials.

There are many other pretherapeutic factors that have affected survival (often used as a parameter of response) as well as objective response rates in gastrointestinal cancer. Table 4 shows the relation of sex and age of patients to the results seen with fluorinated pyrimidine therapy (*3*). Severe leukopenia occurred to a greater statistically significant degree in females, whereas the objective response rate was higher in males. In addition, patients over 70 years of age had a markedly lower response rate when compared with younger patients.



TEXT-FIGURE 1.—Response to chemotherapy and survival prospects for Hodgkin's disease, reticulum-cell sarcoma, and lymphosarcoma.

In all types of gastrointestinal cancer, and especially for colon carcinoma, both median and mean survival are about 100% better for those with regional disease (*3*) only when compared with distant intra-abdominal, hepatic, and extra-abdominal carcinomas (table 5).

Table 6 outlines the relationship of survival to grade of malignancy according to the Broder classification. As expected, the higher the grade of malignancy, the lower the survival. The survival data in this table and in the previous one are on patients at the Mayo Clinic, who were given palliative surgery and maximal supportive care only during a period when they were not receiving radiation therapy and chemotherapy (*3*). Still it is hard to imagine that these would not indicate variables that would affect response to subsequent treatment, including chemotherapy.

Survival, according to the interval between resection of primary carcinoma for cure and proof of recurrence or metastasis (i.e., the so-called "free" interval), is given in table 7. As noted in other diseases, the correlation clearly exists with a nearly 300% difference being observed between extremes in colon cancer.

In table 8, one sees the correlation of the free interval duration to the response to fluorinated pyrimidines. The longer the free interval, the higher the rate of objective regressions.

TABLE 3.—*Results of rapid injection of 5-FU for treatment of advanced carcinoma of the large bowel*[a]

Reporting group[a]	Number of patients treated	Objective regressions, %
Sharp and Benfiel	13	85
Hall and Good	19	63
Rochlin et al.	47	55
Allaire et al.	17	47
Cornell et al.	13	46
Eyerly	12	42
Field	37	41
Bell	22	36
Weiss and Jackson	37	35
Ferguson and Humphrey	12	33
Hurley	150	31
Eastern Cooperative Group	48	27
Brennan et al.	183	23
Hyman et al.	30	20
Ansfield	141	17
Mayo Clinic	144	15
Ellison	87	12
Kennedy	22	9
Knoepp et al.	11	9
Olson and Greene	12	8

[a] *See* (*3*).

TABLE 4.—*Relation of sex and age of patients to results of fluorinated pyrimidine therapy*[a]

Characteristics	Number of patients	Severe leukopenia, %[b]	Objective regressions, %
Sex:			
Male	165	16	22
Female	112	30[c]	15
Age, yr:			
Under 45	26	11	23
45-69	225	22	19
70 and over	26	23	8

[a] *See* (*3*).
[b] <1,500 cells/mm³.
[c] $P<0.01$.

TABLE 5.—*Survival according to extent of gastrointestinal malignant disease* [a]

Extent of disease	Number of patients	Survival, mo	
		Mean	Median
Pancreatic carcinoma			
Regional only	67	8.8	6.0
Distant intra-abdominal (nodes or implants)	27	4.2	3.0
Hepatic	50	3.1	2.5
Extra-abdominal	8	2.5	2.0
Gastric carcinoma			
Regional only	64	10.0	5.0
Distant intra-abdominal (nodes or implants)	120	8.0	4.0
Hepatic	80	5.7	2.5
Extra-abdominal	24	4.1	2.0
Colon carcinoma			
Regional only	104	14.2	12.0
Distant intra-abdominal (nodes or implants)	230	8.3	6.0
Hepatic	242	6.9	5.5
Extra-abdominal	70	6.9	5.0

[a] *See (3).*

TABLE 6.—*Survival according to Broder's grade of malignancy* [a]

Carcinoma	Grade	Number of patients	Survival, mo	
			Mean	Median
Pancreatic	1 and 2	51	8.3	5.5
	3 and 4	94	4.2	3.0
Gastric	1 and 2	25	9.7	7.0
	3 and 4	277	7.4	4.0
Colon	1	118	11.6	10.0
	2	207	10.2	8.0
	3	92	7.9	5.0
	4	62	4.6	3.0

[a] *See (3).*

TABLE 7.—*Survival according to interval between resection of primary carcinoma for cure and proof of recurrence or metastasis* [a]

Carcinoma	Interval, mo	Number of patients	Survival, mo	
			Mean	Median
Gastric	1-5	11	2.4	2.0
	6-11	8	6.1	3.0
	12-23	15	9.1	6.0
	24 and over	21	10.8	6.5
Colon	1-5	29	8.8	4.5
	6-11	27	9.7	8.0
	12-23	42	11.2	10.0
	24 and over	55	13.5	11.0

[a] *See(3).*

Table 9 reveals the importance of the palliative surgical treatment given a primary lesion when it is inoperable. In colon cancer, patients having a palliative resection lived twice as long as those with no surgery performed, and 150% longer than those patients having a bypass. This in itself reflects pretherapeutic selection but is almost surely a factor in influencing response to subsequent therapy.

TABLE 8.—*Relation of clinical aggressiveness of malignant disease to therapeutic results of fluorinated pyrimidines* [a]

Interval from diagnosis of primary cancer to proof of recurrence or metastasis	Number of patients treated	Objective regressions, %
Simultaneous	86	15
1-12 mo	83	18
More than 12 mo	108	25

[a] *See (3).*

TABLE 9.—*Survival according to palliative surgical treatment of primary lesion* [a]

Surgical treatment	Number of patients	Survival, mo	
		Mean	Median
Pancreatic carcinoma [b]			
None	10	5.7	2.0
Gastroenterostomy	20	5.1	4.5
Biliary diversion	67	7.5	4.5
Gastric carcinoma			
None	163	6.9	3.5
Bypass	29	10.0	4.5
Subtotal resection	39	10.0	6.0
Colon carcinoma			
None	100	6.4	4.0
Bypass	131	7.8	6.0
Resection	108	10.9	8.0

[a] *See (3).*
[b] Only patients with carcinoma of the head of the pancreas were considered in this analysis.

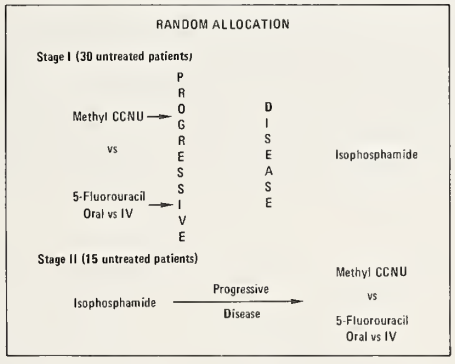
Space does not permit a discussion of other important variables such as morphologic stage, functional stage, pathologic variation, prior radiation and/or chemotherapy, and the response or lack thereof. Clearly, before it is possible to assess a phase II study meaningfully, data must be available on the many pretherapeutic variables, and there must be greater knowledge of the importance of their correlations with response. Therefore, it is not inconceivable that an important drug should undergo several phase II studies in a given tumor type as the major patterns of disease and their interaction with therapeutic responsiveness become more familiar.

Through contract-supported work at the Mayo Clinic, and by a working relationship with the Eastern Cooperative Oncology Group (ECOG) and other cooperative groups, phase II designs utilizing these principles have been developed for large-bowel, pancreatic, and gastric cancer.

The results of pretherapeutic stratification of Moertel and the Mayo Clinic Group for phase II studies in large-bowel cancer are given in text-figure 2. The actual design of one study is shown in text-figure 3. In stage I, a minimum of 30 untreated patients after stratification were randomly allocated to one of the newest nitrosourea drugs methyl-1-3-*cis* (2-chloroethyl)-1-nitrosourea (methyl-CCNU, NSC-95541) or to 5-FU. The 5-FU was further randomized between oral and iv administration. At the time of progressive disease, crossover occurred

1. Site of Primary Tumor
   - A. Rectum
   - B. Colon
2. Status of Primary Tumor
   - A. Unaltered
   - B. Resected
   - C. Bypassed
3. Grade of Anaplasia
   - A. Low Grade (Broder's 1 and 2)
   - B. High Grade (Broder's 3 and 4)
4. Indicator Lesion
   - A. Cutaneous or Subcutaneous
   - B. Abdominal
   - C. Pulmonary
   - D. Hepatic
   - C. Other
5. Age
   - A. <50 years
   - B. 50-65 years
   - C. > 65 years
6. Disease Free Interval
   - A. Simultaneous (< 1 month)
   - B. 1 - 6 months
   - C. 6 - 12 months
   - D. > 1 year

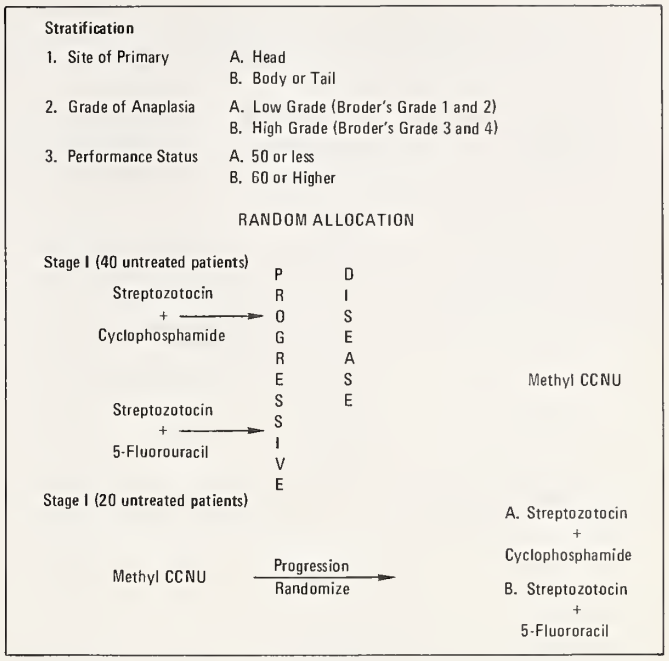
TEXT-FIGURE 2.—Stratification in colon and rectum cancer utilized by the ECOG.



TEXT-FIGURE 3.—Study design for colon and rectal carcinoma.

to a new cyclophosphamide analogue (isophosphamide), which has shown superior activity to the parent compound in several experimental systems. In stage II, a minimum of 15 patients were placed on isophosphamide and, at the time of progressive disease, were randomly allocated to either methyl-CCNU or 5-FU.

This design evaluates three drugs in the therapy of large-bowel cancer. The stratification and random allocation help

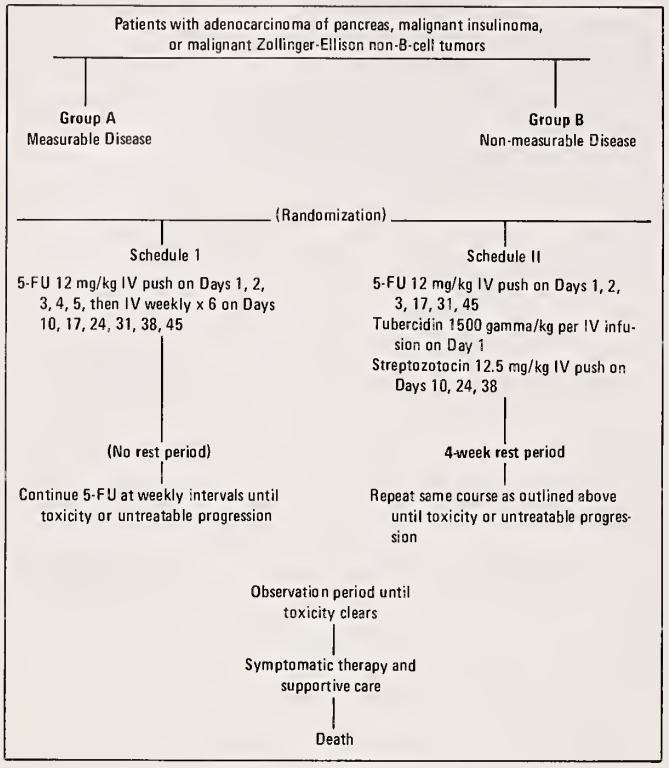


TEXT-FIGURE 4.—Phase II study design for adenocarcinoma of the pancreas.

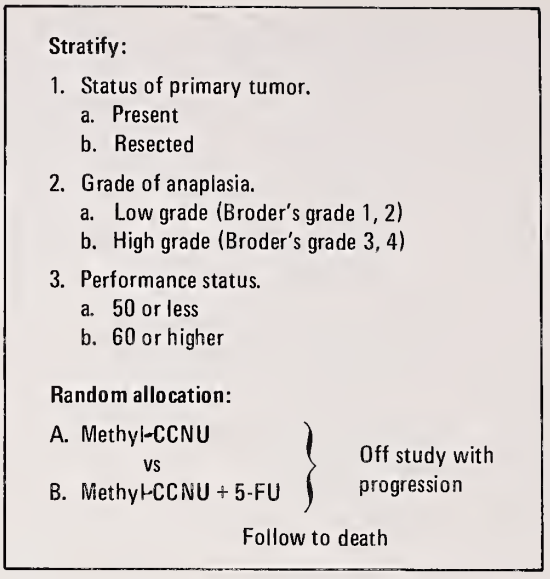
to ensure that the patients treated with the three drugs will be comparable. It should be emphasized that the purpose is not to compare the three drugs in a statistically significant manner, but for the investigators to discern activity that will help them to decide whether further study is indicated, which is the classic question asked by phase II evaluation. In the past, the three drugs would have been sequentially studied in an uncontrolled manner (with the same 45 or more patients), but the data would have been much less reliable due to the potential lack of comparability in these small patient populations. In addition, the crossover design permits gathering of data on clinical cross-resistance between the agents studied at no extra cost in patient population.

A further advantage to this design is that the crossover with the fixed second therapy enables the use of survival as a therapeutic end point with increased validity, as it should reflect the initial therapeutic difference to some degree, although obviously the cross-resistance with the second agent will also be of great importance. This kind of study can measure the percentage achieving standard objective response and duration, and the percentage achieving a status of no change or subjective benefit only. In addition, one can look at a new parameter, i.e., the duration from onset of therapy to progressive disease, which would appear to be a parameter that should be directly related to drug effect (assuming the patients chosen are relatively homogeneous from a pretherapeutic evaluation point of view).

Text-figure 4 outlines the current proposed protocol of the NCI-sponsored ECOG for adenocarcinoma of the pancreas. Here the patients are first stratified by site of primary disease [1) head or 2) body and tail], grade of anaplasia, and performance status. Again, there is a two-stage design with an initial random allocation to drug combinations. Both combinations include streptozotocin, a unique nitrosourea drug without bone marrow toxicity and with marked activity in islet cell tumors of the pancreas (*5*). In one arm, streptozotocin



TEXT-FIGURE 5.—Central Oncology Group protocol for pancreatic cancer.



TEXT-FIGURE 6.—ECOG study for gastric cancer.

will be combined with cyclophosphamide and in the other 5-FU. Since streptozotocin is not myelosuppressive and is limited by renal toxicity, it will be possible to give all the drugs at full therapeutic dose levels in both combinations. At the time of progressive disease, the crossover will be to methyl-CCNU, and possible cross-resistance information will be obtained between two highly different nitrosourea compounds.

In the new protocol for pancreatic cancer by the NCI-supported Central Oncology Group, all histologic types will be used and stratification will be by measurable and nonmeasurable disease (text-fig. 5). Randomization will be to either 5-FU or a three-drug combination of 5-FU, streptozotocin, and tubercidin. Tubercidin is an antitumor antibiotic that has shown some activity in pancreatic cancer but is highly toxic to the veins, and so it must be administered by an in vitro method in which the drug is placed into whole blood and absorbed into the red blood cells. The mixture is then infused into the patient and the drug released by the red blood cells.

Text-figure 6 outlines the ECOG study for gastric cancer in which stratification is by the status of the primary tumor (present or resected), grade of anaplasia, and performance status. Patients after stratification are randomly allocated to therapy with methyl-CCNU or methyl-CCNU +5-FU.

SPECIFIC CONSIDERATIONS IN BREAST CANCER

In the past, phase II studies in breast cancer have been largely uncontrolled and subject to a wide range of interpretation. For example, table 10 summarizes the data of 10 studies on nearly 1,000 patients who received the standard loading dose of 5-FU for treatment of breast cancer. While the overall response rate in 983 patients is 27%, responses range from 20 to 60% in the individual studies. Many explanations are pos-

TABLE 10.—*5-FU given to patients with breast cancer (standard loading dose of 15 mg/kg/day × 5, then 7.5 mg/kg/every other day)* [a]

Investigators[a]	Year	Number who could be evaluated	Number responding	Response, %
Ansfield et al.	1962	676	156	23
Hall and Good	1962	22	15	65
Moore et al.	1968	56	18	32
ECOG	1967	30	8	26
Talley et al.	1965	30	11	36
Field	1963	25	14	56
Eyerly	1962	10	2	20
Vaitkevicus et al.	1961	28	7	25
Kennedy and Theologides	1961	43	18	44
Ravdin and Eisman	1967	63	19	30
Total		983	268	27

[a] *See (6).*

TABLE 11.—*Data from 4 large studies of 5-FU therapy in breast cancer* [a]

Patient selection factors	Study			
	Nemoto and Dao (1971)	Ravdin and Eisman (1967)	Brennan and Talley (1967)	Ansfield (1962)
Previous response to endocrine therapy				
Prior response	3/20 (15)	4/16 (25)	7/17 (41	—
Lack of prior response	9/67 (13)	15/47 (32)	8/45 (18)	—
Post-menopausal status, yr				
0–1	4/16 (25)	8/17 (45)	20/64 (31)	—
1–5	6/41 (15)	3/20 (15)	16/80 (20)	—
5–10	1/23 (4)	5/14 (35)	9/43 (21)	—
Over 10	6/53 (11)	3/12 (25)	13/65 (20)	—
Disease-free interval, yr				
0–2	20/65 (31)	22/50 (44)	—	—
Over 2	16/67 (24)	5/32 (16)	—	—
Site of dominant lesion				
Soft tissue	2/17 (12)	1/6 (16)	22/52 (42)	24/89 (27)
Osseous	0/13 (0)	8/19 (42)	9/51 (18)	67/227 (29)
Visceral	15/203 (14)	10/38 (27)	27/149 (18)	65/360 (18)

[a] *Numbers in parentheses* are percentages.

sible for such divergent results with a single drug and schedule in separate trials in the same tumor including differences in patient selection, intensity of treatment, definition of response, and techniques of data reporting.

Perhaps the most important variable is patient selection. In table 11, the data from 4 large studies of 5-FU therapy in breast cancer show that menopausal status, disease-free interval, and site of dominant lesion all have some correlation with response. Apparently, a woman 0–1 year post menopause with a disease-free interval of 0–2 years and soft-tissue-dominant disease would have a better chance of responding to 5-FU than a woman more than 10 years after menopause with a long disease-free period and visceral-dominant disease. A small uncontrolled series in the latter group, with poor performance status, might show negative results unfairly compromising development of a new drug. In monitoring clinical evaluation of new drugs, which take years to develop, the false negative result is a prime worry for researchers because a negative study is rarely repeated.

The DCT, with its contract-supported institutions (Mayo Clinic, Roswell Park, Sloan-Kettering Institute for Cancer Research, and Albany Medical School), has undertaken development of phase II studies using patient stratification and crossover design of therapy to overcome the problems mentioned above. Patients are stratified on the basis of menopausal status, disease-free interval, and site of dominant lesion. In the first treatment design, patients are randomly allocated to two new drugs that are given in parallel phase II study. When progressive disease occurs, all patients are crossed over to a standard drug or regimen. This design is illustrated in the initial contract study performed by the Mayo Clinic (study A, table 12). Randomization was to CCNU and imidazole mustard with crossover to 5-FU, and the results showed the difficulties of interpreting such a design. Both drugs were only minimally active, and there were no responses in secondary therapy with 5-FU, which allows at least two explanations. Possibly, both drugs were minimally active and cross-resistant with 5-FU, although this is highly unlikely based on experimental and clinical observations with CCNU in gastrointestinal adenocarcinoma. On the other hand, secondary therapy with 5-FU usually produces some response, and it is possible that the patients chosen for study were a selected group with a poor chance of responding to any chemotherapy. This is a disturbing interpretation, even though it is not supported by the results from the Mayo Clinic in recent studies with the same kind of patient population.

In the second design, patients are entered on one new drug or on a standard drug or regimen and crossed to the opposite therapy at the time of progressive disease. Some researchers believe that this design offers the most interpretable data, and

TABLE 12.—*Mayo Clinic phase II clinical trial (study A)*

Therapeutic regimen	Patients for evaluation	Objective responses	Response, %
Initial			
A) CCNU	21	2	9
B) Imidazole mustard	20	2	10
Secondary (5-FU)			
A) After CCNU	14	0	0
B) After imidazole mustard	15	0	0

it is illustrated by the Mayo Clinic study in table 13. The results indicated almost no activity for methyl-CCNU, whereas response to the combination regimen control (Cytoxan + 5-FU + prednisone + vincristine) was in the expected 50% range. The lack of response to the test drug in a population responding adequately to standard therapy offered good evidence that the failure of methyl-CCNU was not a false negative result. This was further supported by the good number of responses when methyl-CCNU failures were crossed over to the combination and by the failure of therapy in the reverse situation.

SPECIFIC CONSIDERATIONS IN BRONCHOGENIC CARCINOMA

The CTEP of NCI has data on over 2,500 cases of bronchogenic carcinoma treated with the standard commercially available antineoplastic agents (table 14). Perusal of the data shows that the alkylating agents (nitrogen mustard or cyclophosphamide) and the antimetabolite methotrexate appear to be the most active agents. Both have the ability to induce an objective shrinkage of tumor masses in about one-third of the cases. The only other compound active in more than 20% of cases is the Japanese antibiotic mitomycin C that has alkylation as its most likely mode of action. It is this degree of activity for single agents that should be kept in mind in evaluating the response data for new agents.

It is common practice to study each new drug in at least 14 patients with a given tumor, because a study with this number of consecutive treatment failures permits rejection of a response rate of 20% with a rejection error of 5% (*2*). Some authors prefer to treat at least 19 patients, because 19 consecutive treatment failures will exclude a 15% response rate with a rejection error of 5%.

The statistical reasoning is impeccable. However, the salvation does not lie in mere numbers. This is illustrated in table 15, in which response rates in different trials of mechlorethamine varied between 0 and 68%. Response in none of the 36 patients (*15*) would have excluded a possible response rate of 9% with a rejection error of 5% (*2*). Thus an overall response rate of 36% in 1,266 patients reported in the literature (*14*) would have been missed.

Classification of the authors into optimists and pessimists is too simplistic an explanation for the wide variation of response rates and does an injustice to their professional competence. Instead, the wide variations of response rates depend, among other things, on factors such as cell type and stage of the tumor, performance and immunologic status of the host, prior therapy, intensity and duration of the present treatment, and criteria for evaluation of objective response. Therefore, it is suggested to log these factors for each patient, to present them in publications of phase II studies, and to pinpoint their prognostic significance in larger studies. Moreover, it might be desirable to use study designs (that permit inclusion of "good-risk patients" into phase II trials without denying them the benefits of chemotherapy of established value), which have an added advantage: They permit comparison of the new drug with a "standard treatment" as a common denominator for response rates. Information on cross-resistance will become available when crossover designs are used. Thus crucial base-line information is gained for larger and more sophisticated comparative clinical trials.

TABLE 13.—*Mayo Clinic phase II clinical trial (study B)*

Therapeutic regimen	Patients for evaluation	Objective responses	Response, %
Initial			
A) Methyl-CCNU	22	1	4
B1) 5-FU, Cytoxan, and prednisone	11	4	36
B2) 5-FU, Cytoxan, prednisone, and vincristine	10	5	50
Secondary			
A) Methyl-CCNU	5	0	0
B1) 5-FU, Cytoxan, and prednisone	6	2	33
B2) 5-FU, Cytoxan, prednisone, and vincristine	10	3	30

TABLE 14.—*Cumulative data on the activity of standard chemotherapeutic agents against bronchogenic carcinoma*[a, b]

Drug	Number of patients treated	Number of responses	Response, %
Nitrogen mustard[c] (Mustargen)	1,123	334	30.0
Cyclophosphamide[d] (Cytoxan)	509	168	33.0
Chlorambucil[e] (Leukeran)	23	3	8.0
Phenylalanine mustard[e] (Alkeran)	38	4	10.5
Busulfan[e] (Myleran)	19	6	32.0
Methotrexate[f] (Methotrexate)	167	56	33.0
5-Fluorouracil[g] (Fluorouracil)	158	12	7.5
6-Mercaptopurine[e] (Purinethol)	95	4	4.0
Arabinosylcytosine (Cytosar)	60	0	0.0
Vincristine[h] (Oncovin)	43	4	9.0
Vinblastine[h] (Velban)	109	12	11.0
Dactinomycin[c] (Cosmegen)	16	0	0.0
Mithramycin[i] (Mithracin)	13	0	0.0
Mitomycin C	135	29	21.0
Hydroxyurea[j] (Hydrea)	36	4	11.0
Procarbazine[k] (Matulane)	172	26	15.0

[a] Compiled by the CTEP, NCI.
[b] *See* (*7–14*).
[c] Merck Sharp & Dohme, West Point, Pa.
[d] Mead Johnson Laboratories, Evansville, Ind.
[e] Burroughs Wellcome Co., Research Triangle Park, N.C.
[f] Lederle Laboratories, Pearl River, N.Y.
[g] Hoffmann-LaRoche Inc., Nutley, N.J.
[h] Eli Lilly & Company, Indianapolis, Ind.
[i] Pfizer Laboratories, New York, N.Y.
[j] E. R. Squibb & Sons, New York, N.Y.
[k] The Upjohn Company, Kalamazoo, Mich.

TABLE 15.—*Objective response of lung cancer to mechlorethamine*

Number of patients		Percent responding	References
Entered	Responding		
36	0	0	(*6*)
26	1	4	(*7*)
58	6	10	(*8*)
61	12	20	(*9*)
43	13	30	(*10*)
21	8	38	(*11*)
41	19	46	(*12*)
54	37	68	(*13*)

Survival as the sole criterion of response (without measurement of tumor size) is a relatively insensitive, time-consuming, and expensive method for determination of response in initial therapeutic trials. Concomitant control groups are imperative, because median survival of successive, staged, placebo-treated control groups, treated by members of a cooperative study group, varied by more than 50% (*16–18*). Hence approximately 20 patients would be needed in each arm of a random allocation study to detect a 100% difference in median survival. The number would increase to approximately 50 patients for detection of a 50% increase in median survival. Corresponding figures for a 20% increase in median survival would be in the neighborhood of 230 patients and, indeed, only 2 of more than 12 agents studied showed superiority over a placebo (*16*). Longer survival of responders might be obscured by shortened survival of nonresponders who are exposed to potentially toxic drugs in the progressive disease.

The study design (text-fig. 7) in lung cancer for phase II trial permits options; the study can be uncontrolled, with inclusion of up to 14 patients who can be evaluated per major cell type and progression to a comparative clinical trial, when antineoplastic activity is recognized. This approach requires admission of patients with a good chance for response.

A controlled approach is preferable, with built-in controls against an unfavorable patient selection. Patients who qualify are allocated at random to one of two treatments: standard (A) or experimental (B). Randomization can be stratified by factors that might strongly modify response (cell type, stage of disease, prior treatment, etc.). Treatment in both regimens is started at a prefixed dose level and schedule. The dose level is then adjusted to individual tolerance (moderate, reversible toxicity). Treatment is continued until progression of disease is noted, which permits cross-correlation between extent and duration of response.

As progression of disease is noted, patients are crossed over; those progressing on drug A will now receive drug B and vice versa. Thus twice as many patients will be needed for the controlled, initial clinical trial as for an uncontrolled one. The crossover design will indicate response rates after prior treatment and might give clues for cross-resistance. A fixed sequence of treatments will also facilitate the use of survival as a parameter for response.

The participants of an International Workshop for the Study of Lung Cancer chose cyclophosphamide; 1,000 mg/m^2/dose given iv every 3 weeks was selected as the standard treatment for all cell types of lung cancer, to be replaced by other standard agents as they proved superior to cyclophosphamide in controlled trials.

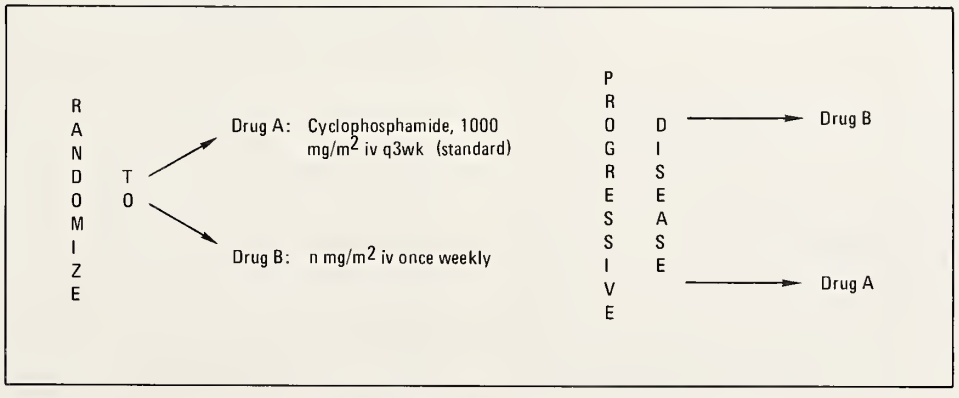
Patient Selection

In a phase II lung cancer protocol, patient selection is critical and each patient should meet all the following criteria:

1) *Microscopically confirmed diagnosis of bronchogenic carcinoma.*—Microscopic diagnosis is based on an adequate biopsy sample or on sputum cytology, permitting classification by cell type, which follows the recommendations of the World Health Organization as advanced by Kreyberg (*19*) and amended at the International Workshop (table 16). The extent of differentiation should be stated for epidermoid carcinoma and adenocarcinoma. Most of the other subclassifications for small-cell carcinoma, adenocarcinoma, and large-cell carcinoma might remain optional until their prognostic implications are elucidated. Some patients, especially those with adenocarcinoma, including the bronchioloalveolar type, will require extensive diagnostic work-up to exclude extrapulmonary primary tumors. Pathology review panels are useful for decrease of observer variability (*20, 21*). Admission of patients with cytologic proof of bronchogenic carcinoma *without* cytologic or bioptic definition of the cell type is not recommended.

2) *Stage of disease.*—The patient must be surgically (or medically) inoperable. Early stages might be more responsive than late ones. Thus 12 of 17 (71%) patients with lymph node metastases responded to CCNU as opposed to 6 of 32 (19%) patients with distant hematogenous metastases (*23*).

3) *Evaluation and measurement of disease.*—Disease that progressed sufficiently to be measured and evaluated was found



TEXT-FIGURE 7.—Study design for phase II trials of new drugs in lung cancer.

TABLE 16.—*Microscopic classification of lung cancer* [a]

World Health Organization [b]	Suggested amendments, including code for data retrieval [c]
I. Epidermoid carcinoma	10. Epidermoid carcinoma 11. Well-differentiated epidermoid carcinoma 12. Moderately well-differentiated epidermoid carcinoma 13. Poorly differentiated epidermoid carcinoma
II. Small-cell anaplastic carcinoma	
1. Fusiform cell type	22. Intermediate (polygonal/fusiform) cell type
2. Polygonal cell type	22. Intermediate (polygonal/fusiform) cell type
3. Lymphocyte-like ("oat cell") type	21. "Oat cell" or lymphocyte-like
4. Others	22. Intermediate (polygonal/fusiform) cell type
III. Adenocarcinoma	
1. Bronchogenic	30. Adenocarcinoma
a. Acinar	31. Well-differentiated adenocarcinoma
b. Papillary	32. Moderately well-differentiated adenocarcinoma
2. Bronchioloalveolar	33. Poorly differentiated adenocarcinoma 34. Bronchioloalveolar
IV. Large-cell carcinoma	
1. Solid tumors with mucin-like content	40. Large-cell carcinoma
2. Solid tumors without mucin-like content	
3. Giant-cell carcinomas	41. Giant-cell carcinomas
4. "Clear"-cell carcinomas	42. "Clear"-cell carcinomas
V.-XIII. Others	50. Others

[a] *See (7–14).*
[b] *See (19).*
[c] *See (20, 22).*

in 82% of 44 patients by Hansen et al. (*24*). Objective appraisal of disease that can be evaluated but unmeasurable is described elsewhere (*25*).

4) *Objective evidence of progressive disease.*

5) *Prior therapy.*—Patients with prior exposure to the study drug or to the same class of drugs are excluded. The same is true for patients with previous radiotherapy to all measurable lesions or those that can be evaluated. All reversible toxicity from prior radiotherapy and chemotherapy must have abated. Most cooperative study groups require an interval of at least 2 weeks between treatments, although intervals of up to 2 months have been recommended (*26*). Exclusion of patients who had received previous treatment other than surgery is optional, based on suggestive evidence for higher response rates in previously untreated patients (*9*). Phase II studies with crossover design should help clarify this point.

6) *Performance status.*—Statements on performance status are optional. Most investigators require an estimated life expectancy of at least 2 months, but predictions are difficult. It is proposed to replace prognostication with performance status to exclude totally bedridden (or bedridden and semiambulatory) patients [table 17; (*26, 27*)].

TABLE 17.—*Evaluation of performance status*

Karnofsky scale [a]		Zubrod scale [b]	
Description	Scale, %	Grade	Description
Normal, no complaints	100	0	Normal activity
Able to carry on normal activities; minor signs or symptoms of disease	90	1	Symptoms but nearly fully ambulatory
Normal activity with effort	80		
Cares for self; unable to carry on normal activity or to do active work	70	2	Some bed time, but needs to be in bed less than 50% of normal daytime
Requires occasional assistance but able to care for most of his needs	60		
Requires considerable assistance and frequent medical care	50	3	Needs to be in bed more than 50% of normal daytime
Disabled; requires special care and assistance	40		
Severely disabled; hospitalization indicated though death not imminent	30	4	Unable to get out of bed
Very sick; hospitalization necessary; active supportive treatment necessary	20		
Moribund	10		
Dead	0		

[a] *See (11).*
[b] *See (9).*

TABLE 18.—*Serial observations (suggested minimum requirements)*

Parameter	Immediate pretreatment and posttreatment	Weekly	Every 2 wk	Every 4–6 wk
Tumor measurement				
Palpable lesion(s)	x	x		
Enumeration of lesions	x		x	
Chest film: posteroanterior and lateral	x		x [a]	
Bone marrow	x			x [a]
Bone scan	x			x [a]
Bone films	x [a]			x [a]
Brain scan	x [a]			x [a]
Host reaction				
Performance	x	x		
Body weight	x	x		
Immunologic status	x			x
Laboratory tests				
Hematocrit or hemoglobin	x		x	
WBC, platelets	x	x [b]		
Creatinine	x	x		
Bilirubin, SGOT, alkaline phosphatase	x	x		
Albumin/total protein	x			x
Na,K, Cl, Ca, P	x			x
Urinalysis	x		x	

[a] In the presence of lesions that could be measured or evaluated.
[b] More frequent with daily or semiweekly drug administration.

7) *Adequate organ functions.*—Criteria depend on toxicity and route of excretion of the study compound. Leukocyte counts greater than or equal to 4,000 or 5,000 cells/mm^3 and thrombocyte counts greater than or equal to 100,000 platelets/mm^3 are required for myelosuppressive drugs. Serum creatinine levels of less than 1.6 mg/100 ml for drugs with renal excretion and bilirubin levels of less than 2 mg/100 ml for drugs with biliary excretion are usually required.

8) *Other primary malignant tumors.*—Concurrent presence of a second malignant tumor excludes the patient because results usually cannot be evaluated.

Evaluation of Response

Quantitation of response in lung cancer is particularly difficult and relates to the tumor, to tumor-related host functions, and to symptomatology. Serial observations should be obtained at appropriate intervals (table 18).

Measurements are obtained with calipers: the product of the longest tumor diameter times the widest perpendicular diameter. Lesions that can be evaluated but not measured require marked and almost complete regression to qualify response. Chest films with lesions should be blinded as to date and patient identification and presented to an expert committee in series of three or more films. Objective response is denoted when there is unanimous agreement on the chest film with the smallest lesion and when this film is obtained after the start of therapy.

Objective tumor response is graded as follows:

1) Complete regression denotes disappearance of all recognizable tumor lesions.

2) Partial regression includes greater than or equal to 50% regression of one or more measurable lesions or marked ($\geq$75%) regression of those that can be evaluated but are nonmeasurable in the absence of progression elsewhere or the occurrence of new lesions. [Some investigators and cooperative study groups define partial regression as $\geq$50% regression of *all* measured lesions in the absence of new lesions. Such a definition appears suboptimal for purposes of a phase II protocol in lung cancer because of the known, significant difference of response by organ site, as established for gastrointestinal cancer (*3*), and because of the difficulty to quantify response of bone marrow lesions.] The use of a category for "improvement," denoting 25–49% tumor regression, is not recommended, because it is difficult to recognize such minor changes on chest films (*25*). Instead, it is desirable to include such patients in the category "static tumor."

3) Static tumor includes changes of less than $\pm$ 50% (<75% decrease or 100% increase for lesions that can be evaluated but are nonmeasurable) in the absence of new lesions. Arrest of tumor growth implies a cell-kill equivalent to new growth and has been shown to relate to survival gain in patients with bronchogenic carcinoma (*28, 29*) and breast cancer (*30*).

4) Tumor progression is documented by any of the following changes: occurrence of any new lesion, irrespective of response elsewhere; increase by more than or at least 50% of a measurable lesion; or more than or at least 100% increase in one to be evaluated. Cerebral metastases are the exception to the rule. Occurrence or progression of cerebral metastases without tumor progression elsewhere is not interpreted as treatment failure because of an assumed blood–brain barrier for most of the commonly used antineoplastic agents.

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Phase III Studies and the Strategy for Integrating Chemotherapy Into a Combined Modality Approach

Stephen K. Carter [1]

For most clinical investigators, the intellectual challenge and excitement in new drug trials center on the drama of translating an antitumor drug from animals to man during phase I and phase II studies. Phase III trials occur much later, but they fill the crucial role of introducing drugs beneficial in the treatment of cancer in the general population.

In classic pharmaceutical terms, phase III studies determine whether an experimental agent will continue to yield the therapeutic results signaled in phase I and II trials. They also reveal unexpected events, such as new types of efficacy and previously undetected adverse effects or interaction with other drugs used to treat certain neoplasms. In essence, the phase III study is still oriented toward the drug and its role in general medical practice. A more recent approach views the drug in the broader context of its use as another tool in the overall therapeutic strategy for each kind of cancer, i.e., a disease-oriented approach combining chemotherapy with other treatment modalities.

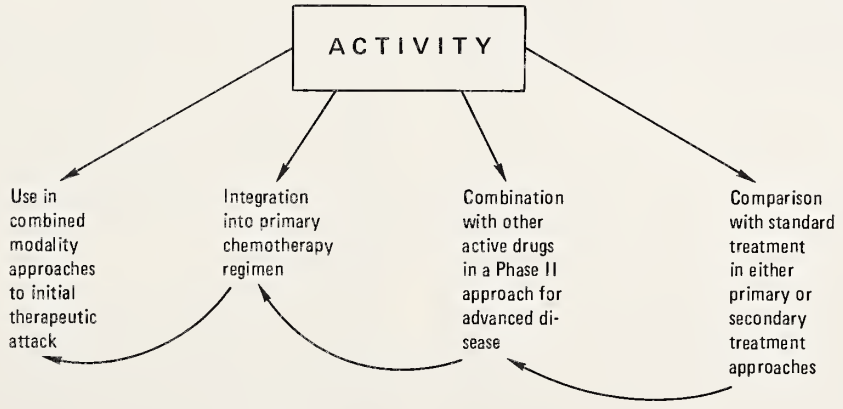
Text-figure 1 depicts a flow scheme for a new active drug with four approaches that can be taken in phase III studies. The most common has been a comparison of the new drug with standard treatment in either the primary or secondary therapy. Another means of studying a new compound is to use it in combination with other active drugs in a phase II study. A third approach is to integrate the new compound into a primary chemotherapy regimen. The last and newest schema is to use the drug in combination with other modalities for initial therapy.

As pointed out earlier, the Division of Cancer Treatment (DCT) of NCI includes within its expanded activities the drug development and clinical testing aspects of the former Chemotherapy Program. The new functions involve the improvement of cancer therapy results through development and clinical evaluation of recent combined modality approaches. These will be developed mainly along disease-oriented lines, with the major emphasis on the solid tumors responsible for most of the cancer deaths in the United States.

The main thrust in the combined modality area will be with chemotherapy plus surgery and/or radiotherapy and/or immunotherapy. This is a logical approach from the standpoint of the established DCT expertise in chemotherapy and because chemotherapy is the only modality of unquestioned effectiveness in killing tumor cells anywhere in the body. Chemotherapy, either with single drugs or combinations, can cure some patients with at least eight different kinds of cancer (*1, 2*).

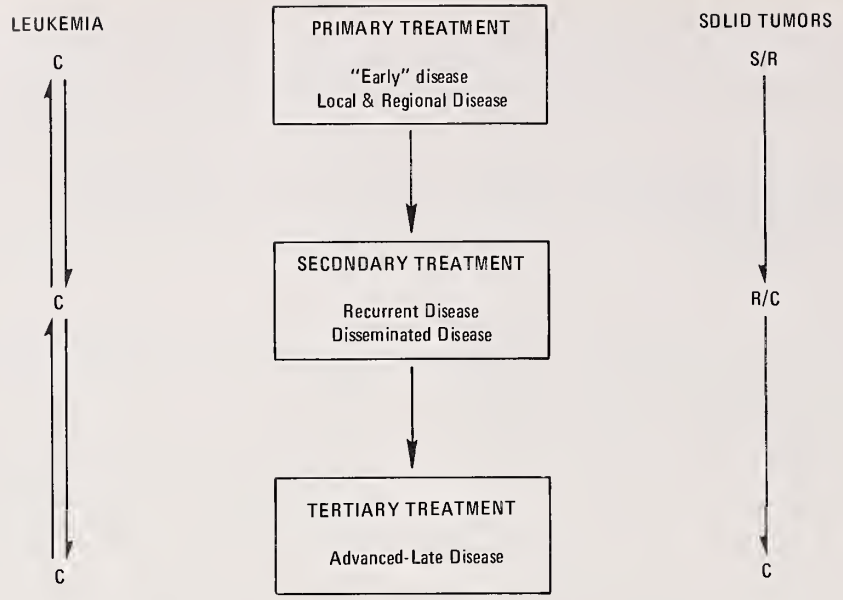
ROLE OF CHEMOTHERAPY IN THE COMBINED MODALITY APPROACH

The philosophical base of the combined modality approach is the recognition that surgery and radiotherapy have reached a plateau in their ability to cure solid tumors. They are local modalities that kill tumor cells only where they are applied; it is not technically feasible to increase the scope of their application in the tumors in which they are effective. They fail to cure many patients, even when they remove all the tumor visible to the naked eye or diagnostic X-ray film. This failure is believed due to the presence of disseminated microscopic disease foci at



TEXT-FIGURE 1.—Flow schema for a new drug active against a given tumor.

[1] Formerly, Deputy Director, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014. *Present address:* Director, Northern California Cancer Program, Palo Alto, Calif. 94304.



TEXT-FIGURE 2.—Comparison of therapeutic modalities in leukemia and solid tumors. C=chemotherapy; R=radiotherapy; S=surgery.

the time of surgical excision of the primary tumor, which many times includes the surrounding tissue and part of the regional lymph nodes.

Chemotherapy, when used optimally, has the potential for eradicating the metastatic foci of early disease. The drug regimens that produce the highest degree of activity in advanced disease will be the prime candidates for use in the combined modality approach. The degree of cell-kill necessary to shrink a bulky solid tumor mass by greater than 50%, which is usually the minimum definition of objective regression, is large. If this level of cell-kill could be directed against the relatively small tumor burden remaining after surgical excision, perhaps eradication of the last neoplastic cell could be achieved.

Experimentally, it is well established that the best chance of eradicating a tumor mass with chemotherapeutic agents is when the tumor cell population is small, a situation occurring at the time of resection of the primary tumor. The effect of chemotherapy in inducing a cure is greater when all visible tumor is surgically enucleated as opposed to subtotal resection (*3, 4*). The inverse relationship between tumor cell population and chemotherapeutic cure was clearly expressed in quantitative terms in mice bearing the L1210 transplanted leukemia (*5*). Similar conclusions have been reached in experimental solid tumor systems (*6*).

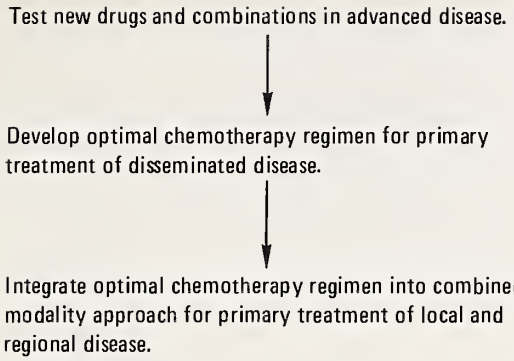
The major successes of chemotherapy have been in the hematologic cancers, especially acute lymphocytic leukemia and advanced Hodgkin's disease (*1*). However, these triumphs of cure and long disease-free survival have not been translated to the common solid tumors that are the major cause of cancer mortality throughout the world. The obvious reasons offered for this disparity of results are the differences in cell kinetics and relative accessibility to effective drug concentrations exhibited by cells in fast- and slow-growing tumors. One additional factor, which is often neglected, is the point in the treatment strategy for a given disease at which chemotherapy is introduced.

As a general rule, the major potential for cure in any tumor is in the initial therapeutic approach. In leukemia, a disseminated disease, chemotherapy is the treatment of choice in all stages (text-fig. 2). The optimum drug regimens are used in primary therapy, and new drugs or regimens are tried in recurrent or advanced disease, with the successful ones being integrated into the primary therapeutic approach.

On the other hand, the primary treatment in solid tumors without disseminated disease is surgery and/or radiotherapy. Chemotherapy is relegated to secondary or tertiary use after the local modalities fail and the disease is advanced and disseminated. Since the secondary or tertiary therapy is rarely curative in any tumor, including hematologic cancers, it is understandable that chemotherapy in solid tumors has not been curative, although it has produced tumor regression, palliative benefit, and some survival gains. Any comparison between the results of chemotherapy in solid tumors and in hematologic ones should take into consideration the differences in the flow of treatment as well as the other dissimilarities between the two tumor types.

The DCT-proposed therapeutic strategy for increasing cure rates in solid tumors involves integration of drugs into combined modalities for primary treatment according to the sequential approach in text-figure 3. In this scheme, new drugs and combinations would be tested in advanced disease and those producing positive results would move into primary treatment of disseminated disease. The optimal regimen evolved in this situation would then be integrated into a combined modality approach for primary treatment of local and regional disease.

Such a proposal for a therapeutic attack against breast cancer is schematically outlined in text-figure 4. The solid arrows show the standard treatment flow in this disease, beginning with mastectomy and moving through hormonal manipulation and eventually to chemotherapy. The broken arrows indicate the concerted efforts of the DCT and the Breast Cancer Task Force that might integrate chemotherapy into the earlier stages of treatment.



TEXT-FIGURE 3.—Proposed steps to increase cure rates in solid tumors with chemotherapy.

THE POTENTIAL OF CHEMOTHERAPY FOR USE IN COMBINED MODALITY THERAPY

An evaluation of the potential of chemotherapeutic agents for use in a successful combined modality thrust in the initial treatment of any tumor requires consideration of two factors: 1) The number of drugs with different mechanisms of action that are active in the advanced stage of the tumor, and 2) the degree of activity exhibited by the active agents alone or in combination.

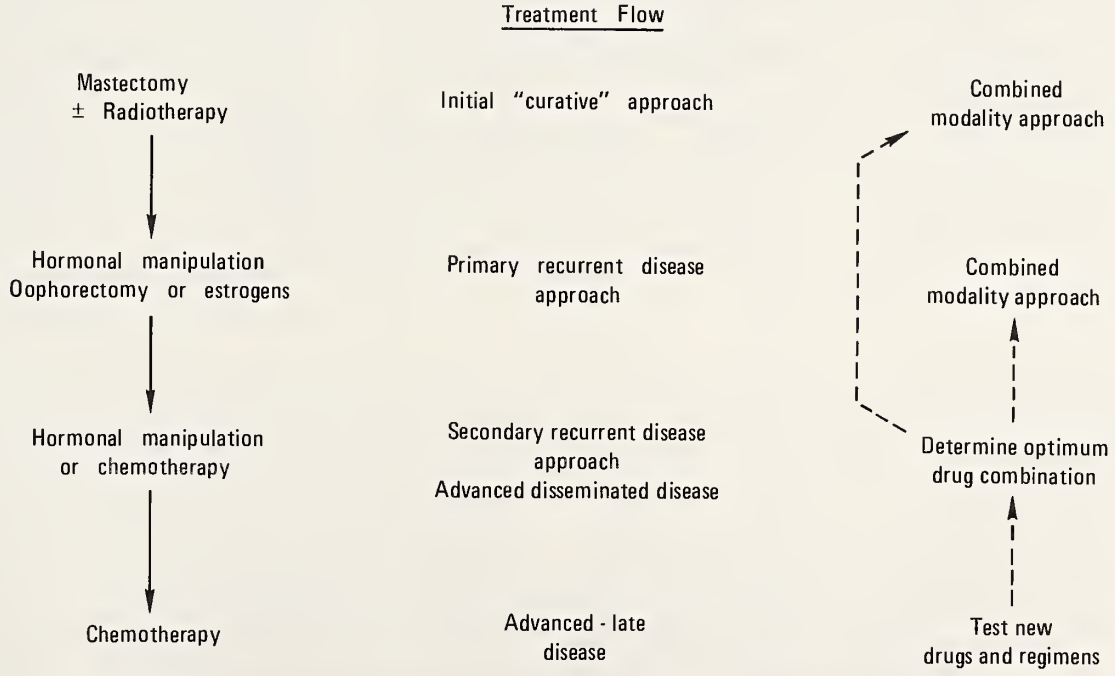
The status of clinical evaluation of 29 drugs against 16 solid tumors based on published (7) and unpublished results on file in the Cancer Therapy Evaluation Program (CTEP) is presented in table 1. Each drug has shown definite evidence of activity against at least one tumor type from among the hematologic or solid tumors. Investigational drugs (which have not been adequately evaluated or were not active in any tumor) and hormonal agents are excluded from this review.

The question of whether the evaluation of a particular drug in a specific tumor has been sufficient to allow a decision on its clinical activity is obviously an arbitrary one. Nevertheless, the drug–tumor interactions in table 1 have been characterized by the staff of the CTEP through analysis of what is considered to be the maximum data available to any single group experienced in evaluating anticancer agents. The designation "NE" denotes drugs that have not been evaluated. Drug–tumor interactions marked "0" refer to situations in which a drug has been inadequately evaluated and no decision can be made regarding its activity. A dash indicates that adequate evaluation has taken place and the drug is inactive. On the other hand, some drugs have been evaluated to an extent permitting a "+" for evidence of activity, although it is not fully established, or "++" when clinical activity is definitely established.

Part A of table 2 presents a disease-oriented analysis of the 16 tumors according to the percentage of the 29 drugs for which a decision on activity is possible. Such a decision on more than one-half the drugs can be made in only 4 tumors; these include the 3 most common (colon, lung, and breast) and 1 uncommon tumor (melanoma) that has been extensively studied because its lesions are readily accessible to measurement and analysis. Among the other 12, the percentage of drugs inadequately evaluated or not evaluated at all ranges from 66% (brain) to 93% (bladder).

When the inadequately evaluated agents are added (table 2, part B), the same 4 tumors head the list. Except for a 4-point drop in rank for pancreatic and a 4-point rise for prostate tumors, there is no significant change in the ranking of the other types. In the remaining 12 tumors, the percentage of unevaluated drugs ranges from 55% (brain) to 83% (pancreas).

Table 3 ranks the solid tumors by the percentage of the drugs tested to a degree establishing definite activity (++; table 3, part A) or evidence of activity (+; table 3, part B) in terms of the total number of drugs adequately studied. Bladder, sarcoma, and stomach lead the list, but relatively few



TEXT-FIGURE 4.—Proposed integration of chemotherapy into combined modality treatment of earlier stages of breast cancer.

TABLE 1.—*Cross-reference chart of drug–tumor interactions*

Drugs	Tumor types															Tumor activity				
	Colon	Lung	Breast	Pancreas	Ovary	Prostate	Stomach	Cervix	Head and neck	Bladder	Kidney	Esophagus	Brain	Testicle	Melanoma	Sarcoma	Spectrum of drugs				
																	NE	0	–	+	++
Alkylating agents																					
Cytoxan	+	++	++	0	++	0	NE	++	–	0	–	0	–	+	–	++	1	4	4	2	5
Nitrogen mustard	–	++	+	NE	0	NE	NE	0	NE	NE	NE	0	–	+	–	NE	7	3	3	2	1
Chlorambucil	–	–	+	NE	+	NE	NE	+	NE	0	0	NE	NE	+	–	+	6	2	3	5	0
Melphalan	–	–	++	NE	++	NE	NE	–	NE	NE	NE	NE	NE	++	–	NE	9	0	4	0	3
Busulfan	NE	–	NE	NE	NE	–	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	14	0	2	0	0
Antimetabolites																					
5-FU	++	–	++	++	++	+	++	++	+	++	–	–	–	NE	–	NE	2	0	5	2	7
Methotrexate	+	++	++	NE	NE	NE	NE	++	++	NE	NE	–	+	NE	–	NE	8	0	2	2	4
6-Mercaptopurine	–	–	–	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	–	NE	12	0	4	0	0
6-Thioguanine	–	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	15	0	1	0	0
Ara-C	–	–	–	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	–	NE	12	0	4	0	0
Mitotic inhibitors																					
Vincristine	–	–	++	NE	–	0	NE	+	NE	NE	NE	NE	–	0	–	++	6	2	5	1	2
Vinblastine	–	–	++	NE	–	NE	NE	NE	NE	NE	NE	NE	–	++	–	NE	9	0	5	0	2
Antitumor antibiotics																					
Dactinomycin	–	–	–	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	++	+	++	10	0	3	1	2
Mithramycin	–	0	–	NE	NE	0	NE	NE	NE	NE	NE	NE	–	++	0	NE	9	3	3	0	1
Daunorubicin	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	16	0	0	0	0
Adriamycin	–	++	++	NE	+	+	NE	+	+	++	–	NE	NE	+	–	++	4	0	3	5	4
Bleomycin	–	–	–	NE	0	NE	NE	+	++	NE	0	–	0	++	–	NE	5	3	5	1	2
Mitomycin C	++	+	+	–	NE	NE	++	0	NE	NE	NE	0	NE	NE	0	NE	8	3	1	2	2
Random synthetics and miscellaneous																					
BCNU	++	+	+	–	0	NE	+	NE	0	NE	0	NE	++	NE	++	NE	6	3	1	3	3
CCNU	++	+	+	NE	NE	NE	0	NE	NE	NE	0	NE	++	NE	+	NE	9	2	0	3	2
Methyl-CCNU	++	+	–	NE	NE	NE	0	NE	NE	NE	0	NE	+	NE	+	NE	5	2	1	3	1
Streptozotocin	–	–	–	–	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	12	0	4	0	0
DTIC	–	–	–	NE	NE	NE	NE	NE	NE	NE	NE	NE	0	NE	++	+	10	1	3	1	1
Hexamethylmelamine	–	++	+	NE	+	0	0	–	–	0	–	NE	NE	NE	–	NE	5	3	6	2	1
Dibromodulcitol	–	–	+	NE	NE	NE	NE	NE	0	NE	NE	NE	NE	NE	0	NE	11	2	2	1	0
Hydroxyurea	–	+	–	NE	NE	NE	NE	0	0	0	–	NE	NE	NE	+	NE	6	3	3	2	0
Procarbazine	–	+	–	NE	NE	NE	NE	NE	NE	NE	NE	NE	0	–	–	NE	10	1	4	1	0
L-Asparaginase	–	–	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	14	0	2	0	0
Dibromomannitol	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	16	0	0	0	0
Drug activity																					
Spectrum of tumors																					
NE	3	3	5	24	18	22	23	17	20	23	15	23	16	18	6	23					
0	0	1	0	1	3	4	3	3	3	4	5	3	3	1	3	0					
–	10	14	10	3	2	1	0	2	2	0	5	3	6	1	14	0					
+	2	6	7	0	3	2	1	4	2	0	0	0	2	4	4	2					
++	5	5	7	1	3	0	2	3	2	3	0	0	2	5	2	4					

[a] NE = not evaluated.
[b] 0 = inadequate evaluation; a decision on drug activity is not possible.
[c] Minus = adequate evaluation; drug inactive.
[d] + = adequate evaluation; evidence of drug activity, but not clearly established.
[e] ++ = adequate evaluation; drug definitely active.

TABLE 2.—*Ranking of tumors on the basis of the percentage of drugs adequately evaluated*

A				B			
Rank	Tumor type	Number of drugs adequately evaluated of total of 29 [a]	Percent	Rank	Tumor type	Number of drugs adequately evaluated of total of 29 [a]	Percent
1	Colon	26	90	1	Colon	26	90
2	Lung	25	86	2	Lung	26	90
3	Breast	24	83	3	Breast	24	83
4	Melanoma	20	69	4	Melanoma	23	79
5	Brain	10	34	5	Brain	13	45
6	Testicle	10	34	6	Cervix	12	41
7	Cervix	9	31	7	Testicle	11	38
8	Ovary	8	28	8	Ovary	11	38
9	Head and neck	6	21	9	Kidney	10	34
10	Sarcoma	6	21	10	Head and neck	9	31
11	Kidney	5	17	11	Prostate	7	24
12	Pancreas	4	14	12	Sarcoma	6	21
13	Stomach	3	10	13	Stomach	6	21
14	Esophagus	3	10	14	Esophagus	6	21
15	Prostate	3	10	15	Bladder	6	21
16	Bladder	2	7	16	Pancreas	5	17

[a] Includes drugs rated −, +, and ++ in table 1.
[b] Includes drugs rated 0, −, +, and ++ in table 1.

TABLE 3.—*Ranking of tumors by percentage of drugs showing definite activity*

A				B			
Rank	Tumor type	Number of drugs with definite activity [a]/ No. of drugs adequately evaluated	Percent	Rank	Tumor type	Number of drugs with evidence of activity or definite activity [b]/ No. of drugs adequately evaluated	Percent
1	Bladder	2/2	100	1	Bladder	2/2	100
2	Sarcoma	4/6	66	2	Sarcoma	6/6	100
3	Stomach	2/3	66	3	Stomach	3/3	100
4	Testicle	5/10	50	4	Testicle	9/10	90
5	Ovary	3/8	37	5	Cervix	7/9	78
6	Cervix	3/9	33	6	Ovary	6/8	75
7	Head and neck	2/6	33	7	Head and neck	4/6	66
8	Breast	7/24	29	8	Prostate	2/3	66
9	Pancreas	1/4	25	9	Breast	14/24	58
10	Lung	5/25	20	10	Lung	11/25	44
11	Brain	2/10	20	11	Brain	4/10	40
12	Colon	5/26	19	12	Melanoma	6/20	30
13	Melanoma	2/20	10	13	Colon	7/26	27
14	Esophagus	0/3	0	14	Pancreas	1/4	25
15	Kidney	0/5	0	15	Kidney	0/5	0
16	Prostate	0/3	0	16	Esophagus	0/3	0

[a] Includes drugs rated ++ in table 1.
[b] Includes drugs rated + and ++ in table 1.

drugs have been evaluated in these tumors. In sarcoma, the fact that every drug tested is designated + or ++ reveals a responsiveness to chemotherapy that should indicate the need for trials with additional drugs. The gynecologic cancers (ovary and uterine cervix) stand out as potentially responsive, but the fact that about 60% of the drugs have not been tested in these tumors should cause concern. Ranking the tumors by the addition of drugs showing activity (+; table 3, part B) further emphasizes the high degree of response in the ovary and cervix. Testicular tumors also rank high, reflecting the known sensitivity of this group to chemotherapeutic agents.

For a drug-oriented view of the available data, each compound is ranked (part A, table 4) with respect to the number of tumors in which adequate evaluation was performed. Only 8 of the 29 agents were adequately tested in more than half the 16 tumors. For the remaining 21 drugs, the percentage of tumors in which evaluation is inadequate or nonexistent ranges from 56% (melphalan) to 100% (daunorubicin and dibromomannitol). If the tumors against which drugs have been inadequately evaluated are included (table 4, part B), 12 of 19 drugs have data of some use in half the tumors. However, this still leaves 17 drugs that have never been evaluated in 56–100% of the tumors.

The top 15 drugs with definitely established activity on the basis of the total number of tumors in which they have been adequately evaluated are listed according to rank (part A,

table 5). 5-Fluorouracil (5-FU) is particularly impressive, exhibiting definite activity in 7 of 14 tumors in which it has received adequate trial. The activity of cyclophosphamide and adriamycin also is noteworthy in a significant number (>10) of tumors. If the drugs are ranked in similar fashion, but including those with evidence of activity as well as definite response, the major change is an upward movement of the nitrosoureas and adriamycin (table 5, part B). This is quite understandable because these are investigational drugs still undergoing clinical trials in phases I and II in some of the tumors.

The major purpose of this review and analysis was to examine the status of clinical drug evaluation in solid tumors as a precondition to identifying the active drugs of potential use in combined modality therapy. Clinical evaluation, from both a drug- and disease-oriented viewpoint, has been generally insufficient and, in fact, data are totally lacking for many drugs and/or tumors. Thus this initial step in the process for integrating chemotherapy into combined modalities is far from complete and emphasizes the need for planning an overall strategy for the rational design of combined modality approaches.

OVERALL STRATEGY OF COMBINED MODALITY THERAPY

The overall strategy for integrating chemotherapy with other modalities of treatment will be disease-oriented and parallel to the sequential approach outlined earlier (text-fig. 3) by consideration of each of the 16 solid tumors individually in a three-stage review and analysis.

Single Agent Chemotherapy

This initial phase will consist of a review of the data to determine which agents have or have not received adequate clinical evaluation in each tumor. Then for the drugs deemed active, an analysis of the degree of antitumor effect and drug toxicity would follow.

This part of the overall strategy will employ data from phase II-type clinical trials (*8*) and include not only a review of existing data but also a determination of the need for further studies. The particular significance of the latter area is quite apparent in the analysis presented earlier (table 2), which shows a lack of definite drug evaluation in most solid tumors. Its importance is emphasized by the fact that the analysis does not include at least 30 investigational drugs and that 7 to 10 new agents enter clinical trials each year.

Despite the enormity of the task, this evaluation of the status of chemotherapy should help to define a therapeutic strategy for each tumor, and perhaps produce a ranking of tumors and drugs similar to the initial efforts made in tables 2–5, which may indicate the drugs most likely to be successful in each tumor and identify the carcinomas most readily approachable by new modalities of treatment. For example, the approach in breast cancer, against which most standard agents have been adequately evaluated (24/29; table 2) and many are active, should be quite different from that in bladder cancer for which only two drugs have received adequate trial.

The findings at this stage should also set the priorities for single-agent, phase II testing in each tumor and delineate them

TABLE 4.—*Ranking of drugs by the percentage of tumors in which they have been at least adequately evaluated or inadequately evaluated a total of 16*

A				B			
Rank	Drug	Number of tumors adequately evaluated	Percent	Rank	Drug	Number of tumors inadequately evaluated	Percent
1	5-FU	14	87	1	Cytoxan	15	94
2	Adriamycin	12	75	2	5-FU	14	87
3	Cytoxan	11	69	3	Adriamycin	12	75
4	Chlorambucil	8	50	4	Bleomycin	11	69
5	Methotrexate	8	50	5	Hexamethylmelamine	11	69
6	Vincristine	8	50	6	Chlorambucil	10	62
7	Bleomycin	8	50	7	Vincristine	10	62
8	Hexamethylmelamine	8	50	8	BCNU	10	62
9	Melphalan	7	44	9	Nitrogen mustard	9	56
10	Vinblastine	7	44	10	Methotrexate	8	50
11	BCNU	7	44	11	Mitomycin C	8	50
12	Nitrogen mustard	6	37	12	Hydroxyurea	8	50
13	Dactinomycin	6	37	13	Melphalan	7	44
14	Mitomycin C	5	31	14	Vinblastine	7	44
15	CCNU	5	31	15	CCNU	7	44
16	Methyl-CCNU	5	31	16	Methyl-CCNU	7	44
17	DTIC	5	31	17	Mithramycin	7	44
18	Hydroxyurea	5	31	18	Dactinomycin	6	37
19	Procarbazine	5	31	19	DTIC	6	37
20	6-Mercaptopurine	4	25	20	Procarbazine	6	37
21	Ara-C	4	25	21	Dibromodulcitol	5	31
22	Mithramycin	4	25	22	6-Mercaptopurine	4	25
23	Streptozotocin	4	25	23	Ara-C	4	25
24	Dibromodulcitol	3	19	24	Streptozotocin	4	25
25	Busulfan	2	12	25	Busulfan	2	12
26	L-Asparaginase	2	12	26	L-Asparaginase	2	12
27	6-Thioguanine	1	6	27	6-Thioguanine	1	6
28	Daunorubicin	0	0	28	Daunorubicin	0	0
29	Dibromomannitol	0	0	29	Dibromomannitol	0	0

[a] Includes drugs rated −, +, ++ in table 1.
[b] Includes drugs rated 0, −, +, and ++ in table 1.

TABLE 5.—*Ranking of drugs based on the number of tumors showing evidence of response or a definite response compared with number of tumors adequately evaluated*

A				B			
Rank	Drug	Number of tumors showing definite response[a]/No. of tumors adequately evaluated	Percent	Rank	Drug	Number of tumors showing evidence of response and definite response[b]/No. tumors adequately evaluated	Percent
1	5-FU	7/14	50	1	CCNU	5/5	100
2	Methotrexate	4/8	50	2	BCNU	6/7	86
3	Cytoxan	5/11	45	3	Methyl-CCNU	4/5	80
4	Melphalan	3/7	43	4	Mitomycin C	4/5	80
5	BCNU	3/7	43	5	Adriamycin	9/12	75
6	Mitomycin C	2/5	40	6	Methotrexate	6/8	75
7	CCNU	2/5	40	7	5-FU	9/14	64
8	Adriamycin	4/12	33	8	Cytoxan	7/11	64
9	Dactinomycin	2/6	33	9	Dactinomycin	3/6	60
10	Vinblastine	2/7	28	10	Nitrogen mustard	3/6	50
11	Vincristine	2/8	25	11	Melphalan	3/7	43
12	Bleomycin	2/8	25	12	DTIC	2/5	40
13	Mithramycin	1/4	25	13	Vincristine	3/8	37
14	Methyl-CCNU	1/5	20	14	Bleomycin	3/8	37
15	DTIC	1/5	20	15	Hexamethylmelamine	3/8	37

[a] Includes tumors rated ++ in table 1.
[b] Includes tumors rated + and ++ in table 1.

according to standard agents or new investigational drugs. It may be much more important to emphasize phase II studies of single agents in bladder cancer than in breast cancer. Also, it may be more meaningful to evaluate standard drugs in bladder cancer rather than new agents.

Combination Chemotherapy

This stage in the overall approach will attempt to integrate the information on single agent chemotherapy into combination drug regimens having maximum cell-kill potential against advanced metastatic disease.

The initial efforts will highlight critical questions concerning the parameters of response to the single drugs active against each tumor, including complete and overall response rate, therapeutic index, duration of response, and impact on survival. It will also be important to consider the differences between the active drugs with respect to mechanism of action, cell-cycle specificity, pharmacologic characteristics, and spectrum of toxic effects. All these factors will play a role in evaluating results with combinations already tested in the clinical setting as well as in examining the drug combinations of future use against each tumor and selecting those with the greatest potential.

Unlike single agent chemotherapy, which developed through predictive evidence of drug efficacy demonstrated in experimental tumor systems, most of the clinical drug combinations have been designed empirically. Nevertheless, the successful regimens are based on the following solid criteria:

1) Each drug in the combination should be active when used alone against the tumor.
2) The drugs should have different mechanisms of action.
3) The toxic effects of the drugs should not overlap so that each can be given at or near its maximum tolerated dose.

As outlined above, a study of these factors will be an integral part of the review and analysis at this stage of the overall strategy. Consideration will also be given to developing retrospective correlations between experimental and clinical results and to investigating new experimental systems that can provide useful data in more rational design of combination regimens. Experimental data may also facilitate selection of the most effective dose ratio, schedule, and sequence of drug administration from among the numerous possibilities inherent in combinations of two or more drugs.

Chemotherapy in Combined Modality Treatment

The final step of the overall strategy will be a review of clinical results that have been achieved by chemotherapeutic agents in conjunction with other treatment modalities. Then these findings and the data obtained in the first two stages will form the basis for integrating the chemotherapy of maximum cell-kill potential against advanced disease into a combined modality approach to primary therapy.

At this point, it should be possible to assess the probable efficacy of combined modalities and set priorities for clinical investigations by weighing the following factors in each tumor:

1) Curative potential of surgery and/or radiotherapy. If all patients could be cured by local modalities, there would be no need for combined therapy. If none are curable by surgery or radiotherapy, the potential of a combined approach may be poor.
2) Cell-kill potential of chemotherapy.
3) Incidence and national impact of the tumor.

For example, at one extreme, breast cancer is the leading cause of cancer deaths in women; most patients are amenable to surgery of curative intent at the time of initial diagnosis, and chemotherapy offers good cell-kill potential. At the opposite extreme, esophageal cancer is relatively uncommon, surgery and/or radiation afford no curative potential, and there is no effective chemotherapy. The other 14 tumors will fall between these two extremes.

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Current Investigational Drugs of Interest in the Chemotherapy Program of the National Cancer Institute

Stephen K. Carter[1] *and* **Milan Slavik**[2]

DRUGS DEVELOPED BY THE DIVISION OF CANCER TREATMENT PROGRAM

Nitrosoureas

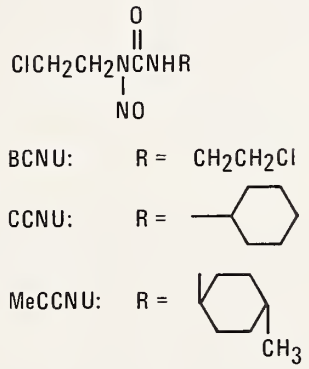
In 1959, work performed under contract by the Southern Research Institute demonstrated weak activity against L1210 by 1-methyl-1-nitroso-3-nitrosoguanidine (MNNG). This compound had a brief but unproductive clinical trial in man. The demonstration of experimental activity led to the evaluation of compounds structurally related to MNNG, and the observation was made that 1-methyl-1-nitrosourea (MNU) was much more effective than MNNG and equally so when mice were given injections of L1210 cells ip or intracerebrally (*1*).

During this same period, results of studies by a group at the Stanford Research Institute (*2*) demonstrated increased activity against L1210 by the 2-chloroethyl and the 2-bromoethyl analogues of MNNG. At the same time, the Southern Research Institute (*3*) was synthesizing congeners of MNU; the 23d nitrosourea evaluated against L1210 was 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU). It was the most active of a number of early congeners examined and was placed in a clinical trial. The activity of BCNU against a broad spectrum of animal tumors has been reviewed (*4*). Almost immediately, clinical activity with this drug was seen, and continued analogue searching was vigorously pursued. This led to eventual clinical trial with 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU, NSC-79037) and recently with 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea (methyl-CCNU, NSC-95441; text-fig. 1).

This historical development of the nitrosoureas points up the rational balance to the initial empirical approach to drug development. The initial demonstration of activity for MNNG was the result of empirical screening, but all subsequent development work on this fascinating group of compounds was the result of rigorous, rationally based, analogue search and the delineation of structure–activity relationships.

Studies on the mechanism of action of BCNU have shown alkylating activity by formation of a diazohydroxide and/or 2-chloroethylamine (*5*), selective interference with the utilization of histidine in 1-carbon metabolism through inhibition of formiminotransferase (*6*), increased NADase activity and decreased NAD^+ (*7*), and decreased DNA nucleotidyltransferase activity (*8*).

Phamacokinetic studies in experimental animals and man



TEXT-FIGURE 1.—Structures of the nitrosoureas.

indicate that BCNU is rapidly metabolized; intact drug is not detected in body fluids shortly after administration. Studies with ^{14}C-labeled drug show prolonged levels of isotope in tissues of monkeys and man, probably representing radioactive fragments of the parent compound. DeVita et al. (*9*) suggest that the breakdown products of the drug may be associated with its delayed toxicity. The radioactive compound is rapidly excreted by mice, which suggests biliary excretion and enterohepatic circulation, but the pattern of excretion is slower in man and monkeys.

BCNU received extensive clinical trial in a variety of tumors, and the compiled data (table 1) of the Cancer Therapy Evaluation Program (CTEP) revealed high response rates in advanced Hodgkin's disease (75/149=50%), brain tumors (34/78=42%), multiple myeloma (12/31=39%), malignant melanoma (17/108=16%), and large-bowel cancer (17/128=13%). In most cases, BCNU was given iv at 200–300 mg/m^2 within 2–3 days. The current recommended dose regimen is 100 mg/m^2/day × 2, iv, with courses repeated every 6 weeks.

Organ toxicities in man include delayed leukopenia and thrombocytopenia, nausea and vomiting, and hepatotoxicity. The few cases of pulmonary toxicity that were reported to the CTEP suggest that the effect may be drug related.

CCNU was the first analogue of BCNU selected for clinical studies because of its superior experimental activity in leukemia (L1210) ascites and great lipid solubility (*10*).

Unlike BCNU, CCNU has an asymmetrical structure that enables identification of its breakdown products (cyclohexylamine, cyclohexylisocyanate, and *N,N'*-dicyclohexylurea). In studies with L1210 ascites leukemia, only CCNU or cyclohexylisocyanate prolongs the S phase to twice the normal period; the other products are inactive (*11*). CCNU inhibition of DNA nucleotidyltransferase activity is about the same as that produced by BNCU (*8*). Recent studies on the binding of the breakdown of ^{14}C-labeled CCNU reveal that radioactivity from

[1] Formerly, Deputy Director, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014. *Present address*: Director, Northern California Cancer Program, Palo Alto, Calif. 94304.

[2] Chief, Investigational Drug Branch, Division of Cancer Treatment.

TABLE 1.—*Summary of BCNU activity against 10 major tumor types*[a]

Tumor type	Number of cases evaluated	Number of patients responding	Response, %
Hodgkin's disease, advanced	149	75	50
Other lymphomas	107	30	28
Brain	78	34	42
Large-bowel cancer	128	17	13
Malignant melanoma	108	17	16
Breast cancer	76	16	21
Bronchogenic carcinoma	83	9	11
Multiple myeloma	31	12	39
Head and neck	25	4	16
Childhood solid	21	4	19
Total	806	218	27

[a]Cumulative data, CTEP, NCI.

cyclohexyl-labeled CCNU is extensively bound to proteins and not to the nucleic acids, whereas radioactivity from ethyl-labeled CCNU is nucleic acid bound, with only a fraction bound to proteins (*12*). These data suggest that CCNU interacts with proteins through cyclohexyl-carbamoylation (*13*) and with nucleic acids by alkylation.

The antitumor activity of CCNU is superior to BCNU in L1210 leukemia, and it is also significantly active against Walker 256 carcinosarcoma in rats and B16 melanoma (*10*).

Pharmacokinetic studies in rodents show that 75% of the ^{14}C-cyclohexyl-labeled or ethyl-labeled CCNU is excreted in the urine in 24 hours after oral or ip administration; about 10–20% of carbonyl- or ethyl-labeled CCNU is expired as $^{14}CO_2$ (*14*). CCNU is rapidly metabolized in dogs and monkeys and excreted predominantly in the urine (*14*). Pharmacokinetic studies with ^{14}C-labeled drug in man show rapid metabolism, prolonged plasma half-life of radiolabeled compounds ranging from 16 to 48 hours, and urinary excretion of 50% of the administered dose within 24 hours and 75% within 4 days with no parent drug detectable in the urine (*15*).

CCNU is perhaps even more active than BNCU in the treatment of Hodgkin's disease (*16*). Table 2 reports the data of Acute Leukemia Group B (Hansen and Selawry) in a controlled study in which BCNU and CCNU were compared. These are the latest data: 54 patients randomly allocated with 36% complete and partial remissions for BCNU and 69% for CCNU. There were five complete remissions for CCNU and only one for BCNU, indicating the superiority of CCNU in this controlled study. Activity was also reported for a wide range of other tumors (table 3) including malignant gliomas (*17*) and gastrointestinal cancer (*18*). The recommended oral dose is 130 mg/m^2 repeated every 6 weeks.

Methyl-CCNU, the newest of the group, was put into clinical trial based on its activity against the Lewis lung tumor (LLT), which was implanted sc [(C57BL × DBA)F_1; BDF_1 mice]. It is considered to be one of the "toughest" tests of a chemotherapeutic agent. Antimetabolites, classical alkylating agents, and other classes of chemotherapeutic agents have little or no effectiveness against this tumor system in the trials performed to date (*10*).

Certain kinetic considerations of the LLT lend it to use as an experimental model system for the preclinical evaluation of drugs in solid tumors. When this tumor reaches about 0.5 g after sc implant, its doubling time is approximately 3 days, and its pulse thymidine index is in the range of 15%. These characteristics should make the LLT a good model for solid tumors in man. Similar labeling indices are found in a host of solid tumors in man.

Pharmacokinetic studies in man after single oral doses of either cyclohexyl-labeled or chloroethyl-labeled methyl-CCNU show rapid absorption of both moieties of the parent compound, with significant plasma levels of radioactivity as early as 10 minutes after administration (*19*). The average peak plasma levels of radioactivity occur at 3 and 6 hours for the cyclohexyl and chloroethyl moieties, respectively. These peak levels correspond to plasma concentrations of between 2 and 4 μg/ml of drug equivalence. The disappearance of radioactivity from plasma for the chloroethyl moiety is single phased with a half-life of 36 hours, whereas the cyclohexyl moiety disappears biphasically with an early exponential phase having a half-life of 72 hours. No parent drug is detectable in any plasma sample.

Phase II clinical trials of methyl-CCNU are proceeding in a number of institutions and cooperative groups. The data accumulated by the CTEP for more than 300 patients indicate methyl-CCNU activity against adenocarcinoma of the colon, malignant gliomas, malignant melanomas, malignant lymphomas, and squamous cell carcinomas at different anatomical sites (table 4). However, the numbers are still small and more data are needed to confirm these findings.

The toxic effects of methyl-CCNU include nausea and vomiting at doses of 170/m^2 or higher and delayed bone marrow toxicity, which is dose limiting (*20*). The recommended oral dose is 200 mg/m^2 every 6 weeks with individual adjustment.

Streptozotocin (NSC-85998) is a broad-spectrum nitrosourea antibiotic originally isolated and purified from a *Streptomyces acromogenes* fermentation broth but it has recently been synthesized (*21, 22*). Structurally, it is composed of a nitrosourea moiety with a methyl group attached on one end and a glucosamine on the other. In both bacterial and mammalian cells, its primary effect is inhibition of DNA synthesis (*23, 24*). In the L1210 system, an exponential cell-kill pattern has been obtained in vitro, which indicates that the drug probably affects all stages of the cell cycle (*25, 26*). Other biochemical studies have indicated that the drug has potent effects on

TABLE 2.—*Comparison of CCNU and BCNU in the treatment of advanced Hodgkin's disease*[a]

Drug	Number of patients who could be evaluated	Number of responses[b]		Responses, %	Median duration of response, days
		CR	PR	CR + PR	
BCNU	28	1	9	36	23
CCNU	26	5	13	69	71

[a]Data of Acute Leukemia Group B.
[b]CR = complete response; PR = partial response.

TABLE 3.—*Summary of phase II Studies with CCNU*[a]

Tumor type[b]	Number of cases evaluated	Number of patients responding		Response, %		
		CR	PR	Total	CR	PR
Breast carcinoma	155	0	18	12	0	12
Colon carcinoma	207	2	18	10	1	9
Bronchogenic carcinoma	185	6	26	17	3	14
Pancreatic carcinoma	18	0	3	17	0	17
Ovarian carcinoma	21	2	4	29	10	19
Malignant melanoma	74	1	9	14	1	13
AML	0	0	0	0	0	0
ALL	2	0	0	0	0	0
Malignant lymphoma						
Hodgkin's disease	50	6	18	48	12	36
Non-Hodgkin's	33	4	5	27	12	15
Malignant gliomas	89	1	39	45	1	44
CLL	2	1	0	50	50	0
CML	0	0	0	0	0	0
Esophagus	19	1	1	10	5	5
Myeloma	3	0	1	33	0	33
Head and neck	31	0	2	6	0	6
Renal cell	54	0	4	7	0	7
Sarcoma	17	0	0	0	0	0
Skin	29	0	5	17	0	17
Gastric	32	1	0	3	3	0
Total	1,021	25	153	17.5	2.5	15

[a] Cumulative data, CTEP, NCI.
[b] AML = acute myelocytic leukemia; ALL = acute lymphocytic leukemia; CLL = chronic lymphocytic leukemia; CML = chronic myelocytic leukemia;

TABLE 4.—*Phase II studies with methyl-CCNU*[a]

Tumor type	Number of cases evaluated	Responses		
		Number of CR	Number of CR + PR	Percent CR + PR
Breast carcinoma	33	0	2	6.1
Colon carcinoma	77	4	12	15.6
Bronchogenic carcinoma	107	1	16	15.0
Pancreatic carcinoma	10	0	0	0
Ovarian carcinoma	5	0	0	0
Malignant melanoma	48	1	9	18.8
Malignant lymphoma	21	1	3	14.3
Malignant gliomas	32	0	9	28.1
Head and neck	25	0	3	12.0
Cervix	4	0	2	50.0
Total	363	7	56	15.4

[a] Cumulative data, CTEP, NCI.

key enzymes involved in gluconeogenesis, as well as an effect on NAD and NADH that may account for its strong diabetogenic action in lower animals (*27, 28*).

Clinically, this drug is unique among the nitrosoureas for its lack of bone marrow toxicity and the prominence of its renal toxic effect, which is dose limiting.

Streptozotocin has shown activity against islet cell carcinoma of the pancreas. The CTEP evaluated 45 case reports of patients with islet cell carcinoma treated with this drug (*29*). Of these, 25 were males and 20 were females with a median age of 54 years; endocrine symptoms occurred in 35 patients and 10 cases were nonfunctional. Streptozotocin was given iv to most patients at a dose of 1–2 g/m^2 weekly; the average total dose was 20 g. Of 35 cases with functional tumors, subjective or objective responses occurred in 28 (80%), and 8 patients died, with a median survival of 16.0 months. Among the 7 nonresponders,6 died with a median survival of 2.0 months. In the 10 cases of nonfunctional tumors, 5 responded objectively.

Of the toxicity that was noted, the most common was gastrointestinal (78% of the patients had nausea and vomiting); renal occurred in 65%, hepatic in 29%, and hematologic toxicity in 11%.

Streptozotocin also had activity in other gastrointestinal malignancies in addition to islet cell carcinoma of the pancreas. In malignant carcinoid, the CTEP collected data on a total of 7 patients with responses in 4; 1 was a complete remission. In adenocarcinoma of the pancreas, 13 patients were treated at various dose schedules, and four partial remissions were re-

ported. Two of these were of short duration (~6 wk) but further studies of this tumor are indicated. Mayo Clinic researchers have found only minimal activity in large-bowel cancer.

Dacarbazine (DTIC)

Dacarbazine, or 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC, DIC, imidazole carboxamide, NSC-45388), is a new anticancer drug developed by the Division of Cancer Treatment (DCT) of the NCI. The structural formula is shown in text-figure 2 (*30*).

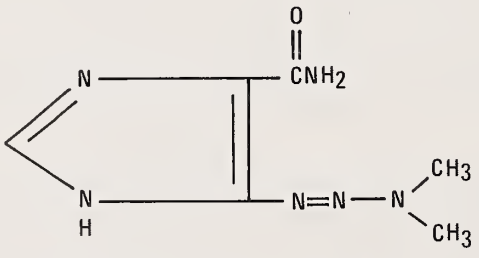
From the inception of clinical trials over 5 years ago, this drug has shown significant activity against malignant melanoma and now is generally considered to be the agent of choice for the treatment of the disseminated form of this disease. The new drug application for dacarbazine has been approved by the FDA, and the compound is now commercially available.

In 1961, the Southern Research Institute synthesized 5-diazo-imidazole-4-carboxamide from 5-amino-imidazole-4-carboxamide and reported its potential antitumor activity (*31*). Because of its instability in aqueous solution, di-substituted and mono-substituted triazenoimidazole-4(5)-carboxamide derivatives were synthesized (*32–34*). Dacarbazine was the most active of these triazenoimidazoles (*35*), and clinical trial was instituted under the sponsorship of the NCI.

Shealy et al. (*35*) reported that dacarbazine significantly increased the average life-span of mice bearing leukemia L1210. Activity was also reported against sarcoma 180, adenocarcinoma 755, and Ehrlich ascites carcinoma in mice (*32*). The activity of dacarbazine against leukemia L1210 on a variety of schedules has been reported by Microbiological Associates Inc., Bethesda, Maryland (*36*) working under contract with DCT.

Wilkoff et al. (*30*) have suggested a 1°-order kinetics model for the killing of L1210 cells by dacarbazine, i.e., the percentage of cells killed at a specific optimal dose is unrelated to the number of cells present. This implies that dacarbazine apparently is not cell cycle, stage specific, and in this respect is similar to alkylating agents such as nitrogen mustard and dissimilar to antimetabolites and cell-cycle-dependent drugs like methotrexate, cytosine arabinoside, 5-fluorouracil (5-FU), and vincristine.

At least three plausible modes of action for dacarbazine have been proposed. Saunders and Schultz (*37*), using a *Bacillus subtilis* model, studied macromolecular synthesis and found that low concentrations of dacarbazine enhanced DNA, RNA, and protein synthesis, whereas high concentrations inhibited DNA formation. Addition of amino acids also enhanced dacarbazine activity. Indeed, the imidazole carboxamides, on purely stereochemical grounds, would be likely agents for deranging de novo purine synthesis. Skibba et al. (*38*) suggest that DIC undergoes *N*-methylation to a monomethyl derivative followed by nonenzymatic formation of an alkylating intermediate (diazomethane), for which a dacarbazine metabolite acts as the carrier. Finally, Saunders and Schultz (*37*) have shown in their bacterial system that dacarbazine inhibition can be reversed by the addition of large amounts of glutathione.



TEXT-FIGURE 2.—Structure of dacarbazine.

Dacarbazine is the most extensively studied drug in disseminated malignant melanoma based on reports from the literature and the files of the CTEP (*39*). Used as a single agent, it has produced more than 50% regressions of tumor in 166 of 758 patients, for a response rate of 22% (table 5).

In the data reported by Luce et al. (*40*) for the Southwest Cancer Chemotherapy Group, there were 21 responses (5 complete and 16 partial) among 110 patients. Of these objectively responsive patients, 9 had metastatic disease in the liver, 7 each in the lungs and skin, and 9 had localized disease only. The complete responses (CR) were seen in 1 patient with cerebral and pulmonary metastases, in 1 with pulmonary and lymph node metastases, in 2 patients with lymph node disease only, and in 1 with skin metastases. The median duration of remission in the responders was 16+ weeks at the last report. In patients who relapsed, the median duration of remission was 17 weeks. Of the patients still alive, survival was 26+ weeks for the responders and 12+ for nonresponders. In those patients who died, survival was 35 weeks for the responders and 12 weeks for the nonresponders.

In an Eastern Oncology Group study comparing dacarbazine dosage in melanoma, patients were randomized to either a high dose (4.5 mg/kg/day × 10) or a low dose (2.0 mg/kg/day × 10) with a second course given after 20 days of observation. Of 43 patients (who met evaluation criteria) given the high dose, 9 obtained partial response status, whereas of 40 on the lower dose, 2 obtained CR and 10 PR status. This would indicate that the low dose is as good if not better than the high dose and would support the uncontrolled findings of the Central Oncology Group that remissions in melanoma began to be seen at 1.0 mg/kg in their phase I study. Mean duration of response in the Eastern Oncology Group study was 16+ weeks for the high dose. Toxicity was somewhat reduced in the low-dose group with only 35% experiencing nausea and vomiting compared with 75% of those receiving the high dose. Leukopenia was also less at the low dose, but none of the patients receiving the high dose had white blood cell counts below 1,500.

The two organ system sites of dacarbazine toxicity frequently encountered are the bone marrow and the gastrointestinal tract. The nadirs of leukopenia and thrombocytopenia observed on the two most commonly used dosage regimens are outlined in table 6. It is important to clarify that Luce's experimental design called for repetition of treatment at 21-day intervals but it was often impossible to re-treat patients until day 28. Since the average nadir was at day 25, the next course of dacarbazine was delayed until recovery from leukopenia and thrombocytopenia was evident. The 10-day treatment schedule of the Eastern Cooperative Oncology Group evidenced an earlier nadir and recovery time. The tempo of the hematosuppression is fairly predictable. The data of the Central Oncology Group, who used a daily × 10 schedule, also suggest this pattern of hematotoxicity.

Luce notes that after an initial dose of 250 mg/m²/day × 5, the leukocyte count was less than 3,000 in 71 of 91 patients. At this same dosage in a group of 83 patients, 8 had platelet counts less than 150,000, 13 were lower than 100,000, and

TABLE 5.—*Dacarbazine in melanoma*

Investigator	Number of patients for evaluation	Number of responses		Response, %	Dosage
		CR	PR	CR + PR	
Central Oncology Group[a]	75		15	20	0.5–5.5 mg/kg/day ×10 days every 28 days
	336	16	58	22	4.5 mg/kg/day ×10 every 28 days
Acute Leukemia Group B[a]	28	2	7	32	300 mg/m²/day ×6 every 15 days
	20	1	5	30	100 mg/m²/every 8 hr ×6 every 15 days
Eastern Cooperative Oncology Group[a]	43		9	21	4.5 mg/kg/day ×10 every 20 days
	40	2	10	30	2.0 mg/kg/day ×10 every 20 days
Luce et al. (*40*)	110	5	16	19	250 mg/m²/day × 5 every 3 weeks
Burke et al. (*41*)	20		4	20	4.5 mg/kg/day ×10
Gottlieb and Serpick (*42*)	12		1	8.3	150 mg/m²/day × 5
	13		2	15	350–450 mg/m² twice weekly
G. Falkson[a]	3			0	100 mg/m²/every 8 hr 18 doses
	12		1	8.3	150 mg/m²/day × 10
	14	2	5	50	300 mg/m²/day ×6
Vogel et al. (*43*)	12		1	8.3	40 mg/m²/day × 5
Cowen and Bergsagel (*44*)	20		4	20	650–1,450 mg/m²
Total	758	28	138	22	

[a] Data on file in CTEP, NCI.

TABLE 6.—*Hematologic toxicity of dacarbazine*

Investigators	Dosage	Mean nadir, day[a]	Recovery, day
Southwest Cancer Chemotherapy Group	200–500 mg/m²/day ×5 days every 21 days	25	40
Eastern Cooperative Oncology Group	4.5 or 2.0 mg/kg/day ×10 days every 20 days	7[b]	20

[a] From initiation of treatment.
[b] Occasionally may be delayed to day 20–24.

only 5 were less than 50,000. The total clinical experience suggests that it is rarely necessary to transfuse patients with blood fractions, but occasionally marrow suppression may be severe and lead to fatality. Often an anemia is noted but it is mild and generally insignificant. There have been infrequent reports of a patient experiencing a severe, unexplainable episode of hematosuppression while being treated with an acceptable dosage (an "idiosyncratic-type" reaction).

Symptoms of anorexia, nausea, and vomiting are the most frequently noted toxic reactions, and over 90% of patients are affected when the initial few doses are administered. The vomiting lasts 1–12 hours and is incompletely and unpredictably palliated with phenobarbital and/or prochlorperazine. Dacarbazine only rarely causes diarrhea. Some helpful suggestions include restricting the patient's oral fluids and food for 4–6 hours before treatment. The rapid toleration of these symptoms suggests that a central nervous system mechanism may be involved; usually symptoms subside after the first day or two. Intractable nausea and vomiting rarely necessitate discontinuance of dacarbazine therapy.

There are a number of minor toxicities that are infrequently noted. Luce described a flulike syndrome of fever to 39° C, myalgia, and malaise in 4 of 202 treated patients. This syndrome occurs 7 days after dacarbazine treatment and lasts 7–21 days, and it may recur with successive treatments. Alopecia was noted in 1 of 13 patients at the Mayo Clinic (*45*); doctors there also described 2 patients with facial flushing and 2 with facial paresthesias.

Malignant melanoma is as notorious for its variable response to therapy as it is for its insidiousness, ease and rapidity of metastatic spread, and fatal outcome. Local irradiation is minimally effective and surgical excision is only occasionally successful in producing prolonged survival. Historically, the results of chemotherapy for malignant melanoma have been most disappointing. Evaluation of results is made more difficult by the fact that spontaneous regression of individual skin lesions is not uncommon. In a few cases, melanoma is responsive to drugs; in many others, it is resistant. As a result, practically every anticancer agent has produced a few clinical responses.

Dacarbazine appears to be the most consistently active chemotherapeutic agent tested against the disease, as is apparent in the comparison with pooled data for the commonly used antitumor agents shown in table 7. Dacarbazine has received

the most extensive testing of any chemotherapeutic agent against malignant melanoma and, in a significantly larger number of patients, has superior or comparable activity to any other agent. The responses with dacarbazine are also remarkably consistent among the various groups studying the drug. This consistency is not seen with agents such as hydroxyurea and dactinomycin, despite what appears to be a significant response rate.

Hexamethylmelamine

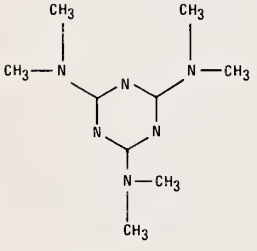
In structure, hexamethylmelamine closely resembles triethylenemelamine (TEM; NSC-9706), a known alkylating agent. A comparison of the two structures is shown in text-figure 3. However, it has been suggested that hexamethylmelamine may not function as an alkylating agent but perhaps as a pyrimidine antimetabolite (*47*), as certain *s*-triazines are known to act. Data on pharmacologic effects in humans suggest that the metabolism of this compound differs significantly from that of TEM.

Heere and Donnelly (*48*) demonstrated that hexamethylmelamine selectively inhibited the incorporation of labeled precursors into RNA and DNA to a greater extent than was observed with the uptake of appropriate protein precursors.

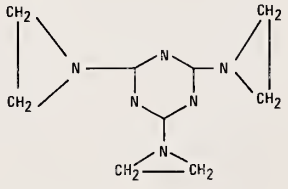
TABLE 7.—*Accumulated data on the commonly used antitumor agents in the single-agent therapy of malignant melanoma* [a]

Antitumor agent	Number of patients who could be evaluated	Number of objective responses	Objective responses, %
Dacarbazine	758	166	22
Nitrogen mustard	34	3	9
Chlorambucil	22	2	9
Cyclophosphamide	37	9	24
Phenylalanine mustard	66	8	12
6-Mercaptopurine	30	2	7
5-Fluorouracil	42	1	2.5
Methotrexate	27	3	11.1
Arabinosylcytosine.	51	1	2
Vincristine	27	3	12
Vinblastine	54	8	15
Dactinomycin	58	19	33
Mitomycin C	8	1	12.5
Mithramycin	13	2	15
Procarbazine	32	6	18
Hydroxyurea	232	57	24
CCNU	81	15	18.5

[a] *See (46).*



Hexamethylmelamine



Triethylenemelamine

TEXT-FIGURE 3.—Structures of hexamethylmelamine and triethylenemelamine.

If the cells were preincubated with the compound, the inhibition of DNA and RNA incorporation of labeled precursors was lost. The authors suggested that this in vitro effect might parallel the in vivo tumor inhibitory effect. Borkovec and DeMilo (*47*) proposed that hexamethylmelamine may demethylate to form the weak alkylator formaldehyde, but this mechanism was presented as a possible alternative. The exact mechanism of action was unknown.

At the maximum tolerated dose (MTD) in the Walker 256 carcinosarcoma system (im) of 80 mg/kg/day, given ip × 10 to 12 days, starting the day after transplantation, the drug inhibited tumor growth by 79%, but it also caused considerable weight loss in the hosts (*49*). The growth of sarcoma 180 was decreased by 25% when hexamethylmelamine was given ip at 125 mg/kg/day × 7 (the MTD). Hexamethylmelamine was inactive against 3-methylcholanthrene-induced rat breast cancer, B16 melanoma, and mouse leukemia L1210.

Bryan et al. (*50*) used an UV spectrophotometric analytic method to study the plasma levels and urinary metabolites of hexamethylmelamine in cancer patients after oral administration. Maximum plasma levels after a single dose of 4 mg/kg were obtained 2 to 3 hours later and averaged 2.75 μg/ml. No intact drug was present in the plasma, but two metabolites were detected. Hemolyzed erythrocytes contained no hexamethylmelamine or drug metabolites. Nearly 19% of a single oral dose was excreted in the urine during the first 24 hours after administration, with an additional 12% appearing between 24 and 48 hours. After prolonged, continual daily administration of the drug, 50–65% of a daily dose was recovered from the urine as metabolites. At least three major metabolites of hexamethylmelamine were found in the urine, but no metabolized compound was recovered.

In mice (*51*), cyanuric acid was the sole urinary metabolite found after the ip injection of ^{14}C-ring-labeled TEM. In the study of Bryan et al. (*50*), cyanuric acid could not be identified as a urinary metabolite of hexamethylmelamine, and they concluded "the ultraviolet absorption and colorimetric behavior of these metabolites suggest that they may retain an *s*-triazine ring structure that does not correspond to cyanuric acid."

Worzalla (*52*) investigated the metabolism of $^{14}C_6$-methylhexamethylmelamine in rodents and man. When the drug was given ip to rats, 30% of the labeled carbon was recovered as $^{14}CO_2$, but if the drug was given orally with a stomach tube, only 13% was recovered in the first 24 hours. Two patients received the labeled material with about 10% of the ^{14}C recovered as $^{14}CO_2$ in the first 6 hours.

When the urine of patients who received hexamethylmelamine was collected, three distinct metabolites were identified chromatographically: N^2, N^4-dimethylmelamine, N^2, N^4, N^6-trimethylmelamine, and melamine. The data suggested that hexamethylmelamine is *N*-demethylated to the lower homologues, and that a significant amount of the methyl groups is removed from the parent compound and oxidized to CO_2.

Hexamethylmelamine has been in clinical trial since 1964, but the lack of absolute comparability of the various patient populations limits the conclusions that can be drawn from the data. Differences in the clinical status of the patients entered into study and the varying methods of evaluation and reporting of the data are responsible in part for this limitation. Legitimate investigator bias, because of the nonrandomized nature of the trials, further hinders conclusions.

Almost all of the investigators have given hexamethylmelamine in divided oral doses of 4–15 mg/kg daily for between 21 and 90 days, but none indicated the superiority of a particular

dose schedule. However, Bergevin et al. (*53*) randomized their patients to either 4 or 8 mg/kg as a daily divided dose for 42 consecutive days. Limiting toxicity resulted in significant dose reductions in the 40 who could be evaluated, but the median dose for the two groups was statistically different (3.5 mg/kg vs. 5.0 mg/kg; $P < 0.006$). Expressed another way, 62% of the patients on the higher dose schedule encountered limiting toxicity compared with 37% of the patients in the lower one.

Bergevin et al. (*53*) observed that the lower the dose being administered, the later the onset of toxicity. They concluded that toxicity was a function of the total dose administered, rather than a direct function of the dose level being administered. Wilson et al. (*54, 55*) compared two dose levels in a retrospective nonrandomized study; therefore, their conclusions that the chronic low-dose schedule resulted in higher overall toxicity were questionable.

The most common toxicity encountered was gastrointestinal and it was often dose limiting. Forty to 70% of the patients vomited to varying degrees, but less than 10% required dose modifications because of the severity of vomiting.

Hematologic toxicity was seen in about 20–40% of the patients evaluated. The onset was usually in the first week of therapy and reached predictable levels. In all patients, the counts returned to their pretreatment levels after the drug was discontinued. The frequency of leukopenia and thrombocytopenia was similar, but leukopenia was more pronounced. There was a low incidence of severe hematologic depression, but no drug-related deaths were reported.

Central nervous system (CNS) toxicity, as manifested by confusion, agitation, and depression, appeared in approximately 10–20% of the patients evaluated. In one report, CNS toxicity necessitated the downward dose modification in 6 of 32 patients being treated. All of the toxic manifestations were reversible, and recovery was usually complete within 4 weeks after the drug had been discontinued.

Weight loss, dermatological reactions, and cystitis appeared infrequently. These toxicities were not serious, nor was their relationship to hexamethylmelamine fully documented.

Table 8 summarizes the therapeutic effect of hexamethylmelamine. For carcinoma of the lung, response rates for the major cell types ranged from 11% for epidermoid carcinoma to 36% for small-cell carcinoma. Adenocarcinoma was intermediate with a 19% response rate. The undifferentiated group, with a 10% response rate, represented patients in whom there was no distinction made between small and large-cell undifferentiated tumors. Response rates of other chemotherapeutic studies were similar, although few reported response by cell type (*56, 57*).

Hexamethylmelamine was evaluated in 32 patients with ovarian carcinoma with 2 complete (6%) and 10 partial responses (31%); this rate was comparable to that of the alkylating agents and 5-FU (*46*).

Uterine carcinoma was evaluated in 6 patients, and 2 of these showed partial responses. Although most antineoplastic agents have not been adequately evaluated in uterine cancer, the potential level of activity of hexamethylmelamine, compared with that of cyclophosphamide and 5-FU, warranted further investigation (*46*).

Seventeen patients with cervical carcinoma were evaluated with hexamethylmelamine therapy with 5 patients responding for a response rate of 29%. This response rate was comparable to that seen with systemic methotrexate (20%), intra-arterial methotrexate (47%), and systemic alkylating agents (20%) (*46*). In 4 patients for whom data were available, the duration of hexamethylmelamine response was 3+, 3+, 4, and 6 months, respectively (*58*); none had had previous chemotherapy.

TABLE 8.—*Summary of hexamethylmelamine single agent activity*[a]

Tumor	Number of patients evaluated	Number of responses		Responses, % (CR + PR)
		CR	PR	
Lung				
Adenocarcinoma	59		11	19
Alveolar	11		0	
Large cell	21		5	24
Small cell	67	7	17	36
Squamous	99		11	11
Undifferentiated	78	1	7	10
Cervix	17		5	29
Ovary	32	2	10	38
Uterus	6		2	33
Bladder	10	1	2	30
Breast	54	4	7	20
Colon	95		11	12
Esophagus	3		1	33
Liver (primary)	8		3	38
Lymphoma	23	2	11	57
Stomach	12		2	17
Head and neck	75		9	12
Kidney	17		1	6
Melanoma	42		2	5
Pancreas	6		0	—
Prostate	6		2	33
Sarcoma	43		1	2
Total	784	17	120	17

[a] Data on file in CTEP, NCI.

Lymphoma patients had good responses in about 57% of the 23 patients evaluated, but the investigators did not specify the duration and degree of response nor the patients' prior chemotherapy. Patients with bladder, breast, colon, and stomach cancer responded at significant levels with responses of 30, 20, 12, and 17%, respectively.

From the 784 patients reviewed in the phase II trials, there are adequate numbers of patients with the following tumor types: adenocarcinoma of the breast and colon, bronchogenic carcinoma, malignant melanoma, and lymphoma. The level of antitumor activity warranted controlled comparative trials in all these tumor types except for malignant melanoma.

In addition, analysis of the data from this review suggested types of responses like those seen for the alkylating agents. This observation, coupled with the biochemical evidence that hexamethylmelamine could perhaps function as an alkylator, points up the need for a clinical evaluation of cross-resistance between hexamethylmelamine and the classical alkylating agents (*47*). If, in fact, there is clinically demonstrable lack of cross-resistance, then hexamethylmelamine may well have a useful role in cancer chemotherapy.

Cis-Platinum (II) Diamminedichloride

This antineoplastic agent is one of a group of platinum compounds first noted to have an antibiotic effect by Rosenberg and his colleagues (*59–62*) and since then was found to exhibit antitumor activity in animals. Structurally, it is a complex formed by a central atom of platinum surrounded by two chlorine or ammonia moieties in *cis*-position (*61*).

Although this compound inhibits incorporation of labeled

precursors of DNA, RNA, and protein in mammalian cells in vitro (*63*), experiments in mice bearing Ehrlich ascites tumor cells indicate a selective inhibition of DNA synthesis (*64*). Interference with DNA synthesis is apparently caused by cross-linking of complementary strands of DNA (*65*).

The drug exhibits antitumor activity in a number of experimental systems, including B16 melanoma in mice, Walker 265 carcinosarcoma in rats (*66*), sarcoma 180 in mice (*62*), and 7, 12-dimethylbenz[*a*]anthracene (DMBA)-induced mammary tumors in rats (*67*). The selection of the drug for clinical studies stems from its significant activity in the L1210 system over a variety of ip dosage schedules. It has no activity by the oral route.[3]

cis-Platinum exhibits synergism in experimental tumors when combined with a variety of anticancer compounds including alkylating agents (*68*), pyrimidine and purine antimetabolites (*69*), 4, 4′-propylenedi-, 2,6-piperazinedione (ICRF 159) (*70*), and *Vinca* alkaloids (*69*).

Pharmacokinetic observations in man show that platinum is rapidly removed from the circulation and widely distributed in the tissues. Less than 10% of the platinum remains in the plasma at 1 hour (*71*). The initial half-life ranges between 41 and 49 minutes, whereas the secondary one is between 58.5 and 73 hours (*71*). About 90% of the plasma radioactivity is protein bound; 19.2–33.9% and 25–43.6% of the administered drug is excreted in urine within 24 hours and 96 hours, respectively (*71*).

Platinum has been used in phase I clinical studies on various dose schedules: single iv dose, repeated every 3 weeks (*71, 72*); daily iv dose × 5 days, repeated every 3 weeks (*72, 73*); and daily doses by iv push until toxicity (*74*). The reported toxic effects include predictable and reversible myelosuppression, reversible renal insufficiency, high frequency toxicity detectable by audiometry, and gastrointestinal intolerance. Researchers doing phase II studies in major signal tumors used dosages of 50 mg/m^2/day, iv, repeated every 3 weeks or 15–20 mg/m^2/day, iv, × 5. Although some activity against malignant lymphomas and solid tumors was reported in the phase I studies, the drug has shown definite activity in germinal cell tumors resistant to previous therapy.

5-Azacytidine

This agent was isolated from a fermentation of *Streptoverticillium ladakanus* (*75, 76*) and independently synthesized by Piskala and Šorm (*77*). The mechanism of action has been elucidated in a number of studies (*78–92*). 5-Azacytidine, an analogue of cytidine, is rapidly phosphorylated and incorporated in both RNA and DNA. By the disruption of the processes of translation of nucleic acid sequences into protein, the synthesis of protein is inhibited. Moreover, it affects de novo pyrimidine synthesis by inhibiting orotidylic acid decarboxylase (*93*).

The drug has exhibited experimental antitumor activity against L1210 leukemia (*94*), lymphoid leukemia in AK mice (*95*), and Ehrlich ascites tumor in mice (*96*). Pharmacokinetic studies in mice indicate that 5-azacytidine is rapidly cleared from plasma, concentrates in lymphatic tissues, and is rapidly excreted in the urine as both unchanged drug and metabolites (*97, 98*). The pharmacokinetics in man indicate a plasma half-life of 3.5 hours after iv administration with 85% of the radioactivity being excreted within 48 hours (*99*).

Phase I clinical studies established the MTD's at 533 mg/m^2, iv, single weekly dose; 160–200 mg/m^2 daily × 5 repeated every 3 weeks (*100*); and 1.6 mg/kg (60 mg/m^2) iv daily for 10 days (*101*). The principal toxic effects are severe nausea and vomiting (apparently dose related) and marrow suppression.

Although activity against solid tumors was reported by Weiss et al. (*101*), it does not appear to be confirmed in phase II clinical trials (*102*). However, promising results were published in the treatment of acute myeloblastic leukemia (*103, 104*) that confirm the original reports from Czechoslovakia (*105*).

L-Asparaginase

This antitumor agent is an enzyme that acts to destroy the circulating asparagine in the peripheral blood and tissues. Certain neoplastic cells lacking asparagine synthetase are thus deprived of a necessary exogenous source of asparagine, and being unable to make their own asparagine, they are required to obtain it from the blood (*106, 107*).

This agent is extremely effective against certain strains of asparagine-dependent mouse leukemias. In patients, asparaginase is useful in the treatment of ALL resistant to conventional agents; when given in doses of from 250 to 100,000 IU/m^2/day, it will produce approximately 50% remissions. L-Asparaginase also exerts some transient inhibitory effect on AML (*108, 109*).

Its particular advantage may lie in the fact that in mouse leukemia it combines well with a variety of agents, such as vincristine, adriamycin, arabinofuranosyl cytosine (ara-C), 5-hydroxypicolinaldehyde thiosemicarbazone (5-HP), and BCNU (*110*). For this reason and its lack of marrow toxicity, it is being studied in many of the protocols in ALL as combination therapy both in induction and in consolidation (*111, 112*). The combinations and diseases for which it will be most effective have not been ascertained.

Guanazole

This compound was introduced into clinical trial based on activity in the leukemia L1210 system (text-fig. 4). Its mechanism of action involves specific inhibition of DNA synthesis, probably through inhibition of the reduction of ribonucleotides to deoxyribonucleotides, rather than suppression of DNA polymerase; therefore, it is the same as described for hydroxyurea but different from cytosine arabinoside, both of which also specifically inhibit DNA synthesis. Guanazole exhibits its greatest activity against L1210 leukemia when administered on a divided dose schedule every 4 days; this implies that relatively high blood levels must be maintained for tissue concentrations to be adequate for a time sufficient to achieve an important level of leukemia cell destruction. In this sense also, it is similar to hydroxyurea and cytosine arabinoside. Animal toxicology studies have shown that guanazole has hepatotoxic and myelosuppressive properties, and it can cause transient hyperglycemia after administration in some species.

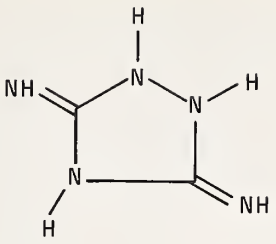
In man, phase I studies have been performed at Roswell Park Memorial Institute and the M. D. Anderson Hospital; a schedule of continuous 120-hour infusions every 14 days based on the experimental data from the L1210 system was used. Doses higher than 20 g/m^2/day × 5 were given by continuous infusion, and definite antileukemic activity was observed; both institutions reported CR in AML. The toxic effects in man have been limited almost exclusively to bone marrow depression, with some fever also being reported.

[3] Venditti JM: Unpublished NCI data.

5-[3, 3-Bis(2-chloroethyl)-1-triazeno]-imidazole-4-carboxamide (NSC-82196)

This compound, known as TIC mustard (text-fig. 5), is an analogue of the dimethyl derivative of dacarbazine (*31, 35, 113, 114*) and has shown clinical activity against malignant melanoma. TIC mustard has marked activity against L1210 murine leukemia, which is clearly superior to that observed with dacarbazine in the same system. In groups of mice treated daily for 30 days beginning 2 days after implantation, there were many 30-day survivors, and the percent of increase in life-span was over 250% at ip doses of 50 and 75 mg/kg/day. Other mice given a single ip dose were also long-term survivors (>240 days at doses of 250, 500, and 750 mg/kg). The schedule-dependency data for this drug in L1210 is presented in table 9.

Initially, TIC mustard was available only in an oral preparation due to its low solubility. Clinical trials revealed this preparation to be irregularly absorbed from the gastrointestinal tract, yielding unpredictable toxic effects. Phase I studies with a parenteral formulation have recently been completed by the Medicine Branch of the NCI and by M. D. Anderson Hospital. The MTD appears to be 900 mg/m^2/day × 5; toxicity effects consist of nausea, vomiting, and bone marrow depression. Some activity, although not dramatic, has been observed in malignant melanoma. In phase II testing in gastrointestinal cancer at the Mayo Clinic, essentially no activity was found; further testing against other tumors is in progress.



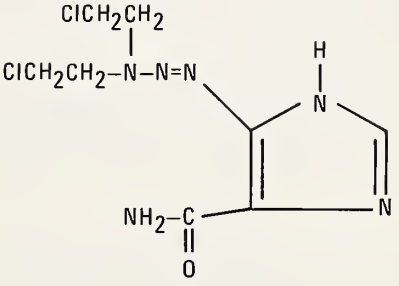
Active System: Leukemia L1210 (optimal schedule Q3h x 8 Days 1, 5, 9)

Mechanism of Action: Inhibition of DNA synthesis through inhibition of ribonucleotide reductase

Dosage: 10-20 g/m^2/day x 5 continuous infusion

Toxicity: Bone marrow depression

TEXT-FIGURE 4.—Structure and basic information for guanazole.



Active System: Leukemia L1210 (highly active on a variety of schedules)

Mechanism of Action: Essentially unknown

Dosage: 900 mg/m^2/day x 5 iv

Toxic Effects: Nausea and vomiting, bone marrow depression

TEXT-FIGURE 5.—Structure and basic information for 5-[3,3-bis(2-chloroethyl)-1-triazeno]-imidazole-4-carboxamide (NSC-82196).

FOREIGN DRUGS CLINICALLY TESTED IN THE UNITED STATES UNDER NCI SPONSORSHIP

New anticancer drugs sponsored for clinical trial by the DCT, NCI, come from various sources, including a program of international liaison for exchanging information on potentially useful antineoplastic agents.

Adriamycin

This drug was originally developed by Farmitalia in Milan, Italy. Clinical studies were initiated in 1969 under the direction of Dr. Gianni Bonadonna of the Istituto Nazionale per lo Studio e la Cura dei Tumori in Milan. The antitumor activity of adriamycin was promptly perceived, and clinical trials in the United States began in mid-1970.

Adriamycin is a glycoside antibiotic. The proposed mechanism of its antineoplastic effect at the cellular level is drug binding to DNA by intercalation between base pairs and inhibition of RNA synthesis by template disordering and steric obstruction. This mechanism of action is based predominantly on data obtained from investigations of daunorubicin (*115*). The evidence for binding of adriamycin to DNA is supported by the ultrastructural changes induced by the antibiotic in mouse hepatic cell nucleoli (*116*).

Wang et al. (*117*) demonstrated that adriamycin inhibited the reaction catalyzed by the DNA polymerase isolated from the L1210 murine tumor cells. Pittillo and Rice (*118*), seeking the exact mechanism of action, were able to show that adriamycin did not inhibit the initiation of DNA synthesis as tested in "thymine-less" death of *Escherichia coli* cells. Bachur (*119*) has been unable to demonstrate the existence of a reductase reaction for adriamycin as he has for daunorubicin.

Experimental tumor studies during 1969 and 1970 revealed the significant antitumor activity of adriamycin and its superiority over daunorubicin in the mouse leukemia L1210 system, the major screening tumor in the drug development program of NCI (*120*). In addition, adriamycin appears to be the most active compound against the B16 melanoma in mice, which is a tumor intensively studied because of its possible kinetic similarity to solid tumors.

Several investigators have stated that adriamycin is rapidly cleared from the plasma of rodents because concentrations of the drug were detected in the liver, spleen, kidney, lung, and heart. Drug excretion is prolonged and occurs predominantly via the liver and kidney (*121, 122*).

Pharmacokinetic studies of adriamycin in man have also demonstrated rapid plasma clearance with a large plasma volume of distribution suggesting a wide drug distribution in

TABLE 9.—*Schedule-dependency data for TIC mustard (NSC-82196) in L1210 leukemia*

Schedule[a]	Optimal dose, mg/kg/day	ILS, %[b]	30- to 40-Day survivors/total treated
ip			
Single dose, day 1	230	>225	5/6
Single dose, day 5	350	>159	1/6
Daily, days 1-5	64	142	0/10
Daily, days 1-30	75	>240	6/6
Every 12 hr, days 1-30	20	>136	1/6
Every 3 days, days 1-30	66	>248	6/6
Every 4 days, days 1, 5, 9	128	228	0/10
Every 7 days, days 1-30	153	>248	6/6
Every 3 hr, day 1 only	256	142	2/10
Every 3 hr, every 4 days, days 1, 5, 9	128	142	0/10
Oral			
Single dose, day 1	1,000	171	5/10
Daily, days 1-5	128	185	1/10
Daily, days 1-30	75	>243	2/6
Every 4 days, days 1, 5, 9	128	164	0/10
Every 3 hr, day 1 only	512	228	9/10
Every 3 hr, days 1-5	64	114	1/10
Every 3 hr, every 4 days, days 1, 5, 9	256	271	7/10

[a] BDF_1 mice are inoculated ip with 10^5 cells on day 1. Control mice (untreated) die between days 8 and 10. Thirty-day survivors are presumed cured.

[b] ILS % = percent increase in life-span over controls.

plasma and tissue. Urinary excretion is prolonged with only 5% of the drug excreted during the first 5 days, which suggests prolonged tissue binding (*123*). Adriamycin is metabolized mostly by the liver, with approximately 50% of the parent compound excreted in bile and an additional 30% excreted as conjugates. Pharmacokinetic studies in patients with hepatic dysfunction show significant and prolonged plasma levels of adriamycin metabolites that are associated with exaggerated clinical toxicity. This observation is the basis of a requirement for dose de-escalation in patients with impaired hepatic function (*124*).

Adriamycin is commercially available in many European countries as well as in the United States.

Adriamycin is administered by rapid infusion; various dose schedules have been investigated. The original studies of Bonadonna were with schedules of 0.4–0.8 mg/kg/day × 3 or 4 days repeated at 4- to 7-day intervals or 20–25 mg/m^2 × 3 days repeated at 3-week intervals (*125–127*). At Memorial Sloan-Kettering Cancer Center, most patients received adriamycin in doses of 0.5–1.0 mg/kg/day for 2–6 days (*128–131*). The most commonly used dose schedule is an intermittent one of either 20–30 mg/m^2/day for 3 days, 20–35 mg/m^2 once every 7 days, or 60–105 mg/m^2 once every 21 days (*132–136*). The currently recommended schedule evolved from clinical experience is 60–75 mg/m^2 given as a single rapid infusion and repeated at 21 days. This recommendation is based on the pharmacokinetics just described and the clinical observations of lower toxicities without apparent loss of therapeutic activity (*126, 135*).

The overall response rate for adriamycin is 26% (46/176) in patients with all types of sarcomas and ranges from 10 to 40% in individual studies (table 10). The variability of response rate as a function of cell type was demonstrated by the findings for bone sarcomas, including a 48% response rate (14/29) in Ewing's sarcoma and 31% (11/35) in osteogenic sarcoma, and soft-tissue sarcomas in which the response ranged from 30 to 36% (*121, 128, 129, 131–133, 135, 137–139*).

Gottlieb et al. (*140*) studied this antibiotic in combination with 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC, DIC), another investigational drug having slight activity against sarcomas. The drugs in this combination are synergistic in L1210 and P388 murine tumor model systems (data of Drug Evaluation Branch, DCT, NCI) (*141*). Clinically, both drugs can be given in combination at doses nearly equivalent to those used for each as a single agent. In good-risk patients, adriamycin is administered at 60 mg/m^2 on day 1 and DTIC is given at 250 mg/m^2/day × 5 days. Of 200 sarcoma patients currently available for evaluation, 85 (43%) are reported as achieving an objective response. Although there are some minor differences in response rates by cell types, not enough cases per cell type have been accumulated to demonstrate significant differences.

Comparison of the efficacy of adriamycin and other drugs in sarcomas is hampered by the relative paucity of data for the standard antitumor agents, which probably reflects the general unresponsiveness of this class of tumors. Friedman and Carter (*142*) reviewed single-drug therapy in osteogenic carcinoma, and the activity of adriamycin (31% response rate) is impressive, in contrast to data collected for other drugs such as mitomycin C (15%), methotrexate (19%), and vincristine with no responses (*142, 143*).

Since the adriamycin studies suggest that tumor response is partially a function of histologic type, it is unfortunate that few data exist for the activity of other agents on the basis of cell type. However, comparison irrespective of cell type reveals that adriamycin has the largest volume of reported data for single-agent trials. Retrospective pooled data show that cyclophosphamide and vincristine are the most active single agents, and the response rate in combination chemotherapy is 24% (*144*). The most important conclusion to be drawn from these comparisons is the real need for well-designed studies of sarcomas by cell type to compare directly adriamycin and more conventional chemotherapeutic agents.

Ample data suggest that adriamycin may be one of the most active single agents against metastatic adenocarcinoma of the breast (*145*).

Reports (*146*) are available demonstrating 44 objective re-

TABLE 10.—*Overall response in adriamycin therapy of sarcomas*

References[a]	Number of patients	Number of responses	Response rate, %
INSCT, Milan (*126*)	39	9	23
MSKCC (*131*)	39	10	26
SWCCSG (*132–134*)	58	17	29
BCRC (*135*)	20	8	40
Mayo Clinic (*136*)	20	2	10
Total	176	46	26

[a] INSCT, Milan = Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy; MSKCC = Memorial Sloan-Kettering Cancer Center, New York, N.Y.; SWCCSG = Southwest Cancer Chemotherapy Study Groups; and BCRC = Baltimore Cancer Research Center, DCT, NCI.

sponses among 121 patients with cancer of the breast (table 11) for a response rate of 36%, which is equivalent to results achieved with such standard agents as cyclophosphamide (34%), methotrexate (33%), and 5-FU (26%). The efficacy of adriamycin is even more impressive in view of the fact that most of the treated patients had failed to respond on combination chemotherapy employing many of the standard agents.

The SWCCSG is now comparing adriamycin with a five-drug combination of cyclophosphamide, methotrexate, 5-FU, vincristine, and prednisone given on either a continuous or intermittent schedule. The preliminary results for adriamycin, with a 48% response (11/23), suggest that it may be as effective as the five-drug combination in regression induction (*145*).

In contrast to the relatively responsive tumors such as malignant lymphomas and pediatric solid tumors, lung cancer is more resistant to chemotherapeutic agents. Clinically useful drug effects reported in lung cancer often include responses that are below the generally accepted criterion of more than 50% objective reduction in tumor mass. For this reason, these lesser responses are included whenever they are reported separately in the data on 229 patients treated with adriamycin (table 12). The response rate of 19%, calculated in the usual manner, increases to 26% when responses of smaller degree are included. These responses range from 0 to 55%.

The effectiveness of adriamycin relative to other single agents can be evaluated on the basis of objective response, but the adriamycin data are insufficient to judge survival as a parameter of response. Within the limitations of historical comparisons, and including the less than 50% regressions, adriamycin ranks among the most effective drugs reported in the data based on over 5,000 patients investigated by Selawry and Hansen (*151, 152*), i.e., mechlorethamine (36%), CCNU (27%), methotrexate (25%), cyclophosphamide (23%), and hexamethylmelamine (20%).

The cell type, which is usually classified by the World Health Organization (WHO) system, is a clinically significant variable in bronchogenic carcinoma (*56, 153*). Response rates by cell type, including any regressions of less than 50%, are available for 164 (72%) of the 229 adriamycin-treated patients (table 13).

Epidermoid carcinoma, which is the most common cell type and affects 42% of all lung cancer patients (*152*), is the most responsive to adriamycin (35%). Large-cell undifferentiated bronchogenic carcinoma, the next most prevalent type (22% of patients), shows a 26% response rate that is similar to mechlorethamine and cyclophosphamide but superior to hexamethylmelamine and methotrexate. The remaining cell types, adenocarcinoma and small-cell carcinoma, seem comparatively less responsive.

Adriamycin is active in bronchogenic carcinoma, especially in the major cell types. This conclusion is based on retrospective comparison with other drugs in populations that may not be comparable. A final judgment of the activity of adriamycin vis-à-vis other active agents must await prospective controlled trials.

A total of 147 patients with malignant lymphomas have been treated with adriamycin. The response rates in invididual studies ranged from 32 to 53% with a total response rate of 41% (61/147). Nine patients had complete regressions (table 14).

Most of the patients were treated before the widespread use of the Rye and Rappaport classification systems (*154, 155*). Analysis of results with the older nomenclature is possible for 133 patients as follows: 36% response rate (23/64) in Hodgkin's disease, 56% (19/34) in reticulum cell sarcoma, and 34% (12/35) in lymphosarcoma (*126, 131, 133, 135*).

Adriamycin could be considered inferior by retrospective comparison with most other active agents (*156–158*). However, most of the adriamycin-treated patients had advanced disease and probably had failed standard therapy including combination chemotherapy. In this type of patient population, the response rate observed could be significant because it sug-

TABLE 11.—*Activity of adriamycin compared with standard agents in advanced breast cancer* [a]

Drug (references) [b]	Number of patients	Number of responses	Response rate, %
Adriamycin			
INSCT, Milan (*126*)	19	3	16
MSKCC (*131*)	3	0	—
BCRC (*135*)	4	2	—
SWCCSG (*145*)	95	39	41
Total	121	44	36
Cyclophosphamide (*146*)	529	182	34
Methotrexate (*146*)	356	120	30
Thio-TEPA (*146*)	162	48	30
5-Fluorouracil (*146*)	1,263	324	26
Vincristine (*146*)	226	47	20

[a] Agents for which data are available on more than 100 cases.
[b] *See* footnote, table 10.

TABLE 12.—*Adriamycin activity in bronchogenic carcinoma* [a]

References [b]	Number of patients	Number of responses		Response rate, %	
		<50%	>50%	<50% + >50%	>50%
MSKCC (*131*)	8	—	0	—	0
SWCCSG (*133*)	44	—	7	—	16
BCRC (*135*)	9	—	5	—	55
Praga (*147*)	50	6	3	18	6
Kenis (*148*)	50	—	16	—	32
INSCT, Milan (*126, 149*)	37	10	5	38	11
RPMI (*150*) [c]	31	—	9	—	29
Total	229	16	44	26	19

[a] — = not reported.
[b] *See* footnote, table 10.
[c] RMPI = Roswell Park Memorial Institute, Buffalo, N.Y.

TABLE 13.—*Single drug chemotherapy of bronchogenic carcinoma by cell type*[a]

Cell type	Drugs[b]	Number of patients	Response rate, %
Epidermoid	Adriamycin	57	35
	CCNU	29	41
	Cyclophosphamide	183	19
	Mechlorethamine	111	33
	Methotrexate	140	25
	Procarbazine	30	20
Large cell undifferentiated	Adriamycin	27	26
	Cyclophosphamide	22	23
	Hexamethylmelamine	37	17
	Mechlorethamine	129	27
	Methotrexate	104	12
Adenocarcinoma	Adriamycin	40	25
	CCNU	21	24
	Hexamethylmelamine	34	20
	Mechlorethamine	80	29
	Methotrexate	25	32
Small cell	Adriamycin	40	25
	Cyclophosphamide	120	50
	Hexamethylmelamine	67	36
	Mechlorethamine	51	39
	Methotrexate	20	50

[a] Includes only drugs evaluated in 20 or more patients.
[b] Adriamycin data (*133, 148–150*); data on all other drugs (*151, 152*).

TABLE 14.—*Adriamycin activity against lymphomas*

References[a]	Number of patients	Number of responses	Response rate, %
INSCT, Milan (*126*)	63	25 (7 CR)	40
MSKCC (*131*)	22	7	32
SWCCSG (*133*)	47	21	44
BCRC (*135*)	15	8 (2 CR)	53
Total	147	61 (9 CR)	41 (6% CR)

[a] *See* footnote, table 10.

gests a low level of cross-resistance between adriamycin and other agents. Thus adriamycin should be considered for use in patients refractory to other drugs and for inclusion in new combined therapy approaches. Although supporting data are not available, adriamycin might be highly active in previously untreated patients.

Of 44 patients with neuroblastoma treated with adriamycin, 16 (36%) had objective responses (*126, 131, 133, 138, 159*). Neuroblastoma is predominantly a pediatric tumor, but analysis by patient age, a known clinical variable, is not available. The level of adriamycin activity, although inferior to cyclophosphamide and vincristine (*46, 160*), is substantial when it is considered that the treated patients were advanced cases who had failed other therapy, including cyclophosphamide and vincristine.

Adriamycin has been administered to 195 patients with refractory acute leukemia, and 24% (47/195) achieved CR (table 15).

By cell type, 36 of 148 (24%) patients with ALL had a complete response and the overall rate was 39% (58/148), whereas those with AML exhibited a 23% CR rate (11/47) and an overall response of 32% (15/47). Most of the leukemic patients treated with adriamycin had advanced disease. Survival data were not reported.

The determination of the relative efficacy in ALL of adriamycin, either as a single agent or in combination chemotherapy, compared with other compounds is of low priority in view of the high induction rates achieved by vincristine and prednisone (*162*). For AML, there is little probability that remission induction by adriamycin would be greatly different from the established activity of daunorubicin (*163*). However, a comparison of the activity of the 2 drugs and (more importantly) their relative toxicities may be worthwhile.

The data for testicular tumors (predominantly "nonseminomatous") treated with adriamycin (table 16) show an overall response rate of 18% (7/39).

The objective response rate is the only parameter for which enough data exist for a comparison of adriamycin with other agents. Based on the results with agents used in more than 25 patients, the response to adriamycin is markedly inferior to five other drugs. However, since the adriamycin-treated patients had advanced disease after failure of therapy with drugs of

TABLE 15.—*Adriamycin activity in acute leukemias*

Tumor (references)[a]	Number of patients	Number of responses		Response rate, %	
		PR	CR	PR + CR	CR
ALL					
INSCT, Milan (*126*)	3	1	2	—	—
MSKCC (*131*)	47	13	5	38	11
BCRC (*135*)	5	0	0	—	—
RMPI (*138*)	30	1	5	20	17
Mathé et al. (*161*)	63	7	24	49	38
Total	148	22	36	39	24
AML					
MSKCC (*131*)	6	1	2	—	—
RPMI (*138*)[b]	14	0	1	7	7
Mathé et al. (*161*)	27	3	8	41	30
Total	47	4	11	32	23

[a] *See* footnote, table 10.
[b] *See* footnote *c*, table 12.

known activity, the data suggest significant activity by adriamycin and a low level of cross-resistance with agents currently in use against testicular neoplasms (*164*).

The present thrust of treatment in metastatic testicular cancer is combination chemotherapy. The lack of cross-resistance, with or without future demonstration of greater response in earlier stages of the disease, indicates a need for further evaluation of adriamycin in a single-agent, comparative trial and in combined modality therapy.

Adriamycin has a 25% (5/20) overall response rate in ovarian cancer (*131, 133*). Although ovarian cancer is a relatively responsive tumor, showing 30–50% response rates with chlorambucil, melphalan, cyclophosphamide, hexamethylmelamine, 5-FU, and 5-fluorodeoxyuridine (floxuridine, 5-FUDR) (*156, 165*), adriamycin appears active in view of the fact that most of the treated patients had not improved with other therapy. The observation of approximately equivalent responses to three classes of antitumor compounds might provide a lead for obtaining greater response through drug combinations.

Adriamycin exhibits antitumor activity in bladder cancer, producing an overall response rate of 33% (17/52). Based on published reports, 5-FU (35% response; 26/74) is the only other agent that has been adequately evaluated (*46*). Although adriamycin is active, there are insufficient data for meaningful comparison with other drugs. The highest priority should be placed on properly designed comparative trials of other standard agents. With its demonstrated activity, adriamycin will probably become a standard agent in bladder cancer even without adequate comparative data.

Excluding bronchogenic carcinoma, adriamycin has been evaluated in 44 patients with squamous cell carcinoma for an overall response rate of 23% (10/44) and a range of 15–45% in the different studies (*126, 131, 133*).

In an analysis by anatomic site, this antibiotic had a significant level of activity against squamous cell carcinoma of the cervix. Although only 10 patients have been reported in the literature (*131, 133*), unpublished summarized data show nine responses in 28 patients (32%) treated. The activity of adriamycin is in the range of other active compounds such as methotrexate (*46*). Comparative single-agent trials and/or combination chemotherapy studies with this drug are warranted.

The overall response in head and neck squamous cell carcinomas (*126, 131, 133*) is 23% (8/34), which is apparently inferior to that obtained with other active agents like methotrexate, cyclophosphamide, and bleomycin (*46, 157*). However, it might be of some value in combination-drug regimens.

Adriamycin has been studied more extensively than any other antineoplastic agent in thyroid cancer. Thus far, the response rate is 45% (10/22) but analysis by cell type is not available. The review of chemotherapy in thyroid tumors by Gottlieb et al. (*166*) points out the limited accounts reported in the literature. Adriamycin could be considered the drug of choice for progressive metastatic thyroid cancer on the basis of its known activity and the lack of positive data for other agents and could become the reference drug for trials of more conventional chemotherapeutic agents.

The SWCCSG reported two responses to adriamycin in 9 patients (21%) with adenocarcinoma of the prostate (*133*). This activity is within the range observed for other agents, all of which have been incompletely studied (*167*).

The SWCCSG obtained two responses with adriamycin in 21 advanced myeloma patients who had failed other therapy (*133*). The response rate (10%) in this refractory population may be significant in comparison with pooled data for standard agents such as melphalan, cyclophosphamide, and prednisone (*156*). Impaired renal function, relatively common in advanced myeloma, is not a contraindication to adriamycin.

Gastrointestinal carcinoma is particularly resistant to chemotherapy. The 9% response rate (5/58) achieved with adriamycin is low but, when viewed in the context of an advanced status of disease in treated patients, the drug may not be significantly inferior to the commonly used 5-FU, mitomycin C, and BCNU (*156*). A final conclusion on the activity of adriamycin awaits the results of ongoing studies.

Few data are available on the use of chemotherapeutic agents in metastatic adenocarcinoma of the uterus. However, the SWCCSG reported three responses in 8 patients treated with adriamycin (*133*). Interpretation of this finding is limited by the small number of patients evaluated on other drugs.

The general toxic effects induced by adriamycin are dose related, predictable, and reversible. The major toxicities are dose-limiting myelosuppression in approximately 60–80% of the patients, stomatitis in as many as 80%, nausea and/or vomiting in 20–55%, and alopecia in virtually all cases (table 17).

Leukemia is the predominant manifestation of hematologic toxicity and the severity depends on the adriamycin dose and the regenerative capacity of the bone marrow. In 32 patients with breast cancer without prior myelosuppressive therapy who received 60 mg/m^2 of adriamycin every 21 days, 70% developed leukocyte counts of less than 3,000/mm^3 and 4% had counts less than 1,000/mm^3. By contrast, among 45 patients with previous myelosuppressive therapy who were treated with 60 mg/m^2 ("adequate") or 45 mg/m^2 ("inadequate") marrow, 83 and 15% had counts of less than 3,000 and 1,000/mm^3, respectively (*145*). The nadir of leukopenia generally occurs during the second week of therapy and normal peripheral counts return by the fourth week (*132, 133, 135*). Thrombocytopenia and anemia occur in the same time frame as leukopenia but they do not represent as great a problem. Supportive care for hematologic problems should be available for patients being treated with adriamycin.

Drug-induced stomatitis typically begins as a burning sensation with erythema of the oral mucosa, which in 2–3 days may produce frank ulcerations particularly in the sublingual and lateral tongue margins. Retrospective comparison of the incidence of stomatitis as a function of dose schedule suggests that

TABLE 16.—*Activity of adriamycin compared with other agents in testicular tumors*[a]

Drugs (references)[b]	Number of patients	Number of responses	Response rate, %
Adriamycin			
MSKCC (*131*)	2	1	—
SWCCGS (*56, 132*)	12	2	17
BCRC (*135*)	5	1	20
INSCT, Milan (*164*)	20	3	15
Total	39	7	18
Melphalan (*156*)	86	49	57
Dactinomycin (*156*)	31	16	52
Vinblastine (*156*)	25	17	68
Mithramycin (*156*)	305	113	37
Bleomycin (*156*)	38	12	31

[a]Adriamycin is compared only with data published for agents used in more than 25 patients.
[b]*See* footnote, table 10.

TABLE 17.—*Adriamycin toxicity*

References[a]	Number of patients	Patients exhibiting toxic effects, %				
		Myelosuppression	Stomatitis	Nausea and/or vomiting	Alopecia	EKG changes
INSCT, Milan (*126*)	96	84	79	36	90	30
MSKCC (*131*)	200	60	75	55	85	26
SWCCSG (*132*)	67	—	41	21	100	6
SWCCSG (*133*)	409	75	Occasional	—	Nearly all	8
ALGB (*138*)[b]	86	77	70	45	100	7

[a] *See* footnote, table 10.
[b] ALGB = Acute Leukemia Group B.

it may be less frequent as the interval increases between doses (*126, 134*).

Benjamin et al. (*123*) demonstrated the importance of dose reduction in patients with liver disease. This recommendation is based on pharmacokinetic studies showing prolonged adriamycin plasma half-life and lower biliary excretion in patients with impaired hepatic function. Furthermore, their retrospective analysis showed more pronounced drug toxicity in patients with liver disease who received full doses of adriamycin.

Alopecia involving the scalp, axillary, and pubic hairs occurs in almost all patients. Growth usually resumes on cessation of drug.

Gastrointestinal (GI) toxicities evidenced by nausea and occasional vomiting are associated with the drug but rarely limit clinical use. For example, only 4 of 404 patients in a large cooperative group study refused further therapy because of GI effects (*134*).

Extravasation during iv administration can produce local tissue necrosis, but normal precautions can prevent this toxicity.

The potential cardiac toxicity, which may involve transient EKG abnormalities and/or definite myocardiopathy, is a most important consideration in the use of this drug. Electrocardiographic changes associated with adriamycin therapy are reported in 6–30% of the treated patients (table 17). These generally transient abnormalities, including supraventricular tachyarrhythmias, ventricular extrasystoles, and ST–T wave changes (*131, 133, 139, 168*), occur most frequently in the first few days after drug infusion. On occasion, further drug therapy is withheld until the EKG returns to pretreatment configuration. To date, no significant morbidity or mortality due to the transient EKG changes has been encountered, and there is no evidence that these changes are dose or schedule dependent. Correlation of previous heart disease and EKG abnormalities with adriamycin therapy has not been adequately analyzed.

In contrast to the transient EKG changes, drug-induced myocardiopathy produces significant morbidity and mortality. This "pump" failure is dose dependent but shows no apparent relationship to preexisting heart disease. The clinical presentation and pathophysiology of cardiac damage by adriamycin is indistinguishable from other known cardiomyopathies (*169*). Although the speed of the clinical course varies, it is most often a rapidly progressing syndrome of congestive heart failure and cardiorespiratory decompensation including dilation of the heart, pleural effusion, and venous congestion (*168*). Reversibility of the heart failure does not appear to be a function of the therapeutic intervention used. In fact, Gilladoga et al. (*170*) report that adriamycin cardiomyopathy may be reversed by conventional medical management.

The pathologic findings are limited to changes visible by electron microscopy, the most dramatic of which is a marked decrease in the number of myocardial fibrils accompanied by mitochondrial changes characterized by swelling, focal membrane thickening, and dense inclusions. Other observations include nuclear degeneration, disorganization of the sarcoplastic reticulum, and depletion of glycogen granules (*168*). These changes are nonspecific and have been described in other types of cardiomyopathy (*171*).

The overall incidence of congestive heart failure due to drug-induced cardiomyopathy, based on all available data, is 22 (1%) of 1,934 treated patients. The analyses of the data from M. D. Anderson Hospital and that of the SWCCSG by Gottlieb et al. (*172*) show an incidence of nonfatal and fatal cardiomyopathy of 0.4 and 1.2%, respectively. The interval between the last adriamycin dose and congestive heart failure was 1–6 months (median 2.5 months) and development of cardiomyopathy was dose dependent. In patients receiving less than 500 mg/m^2 as a total dose, the incidence was negligible, but it became markedly higher at a total dose above 550 mg/m^2 (30%) as depicted in table 18.

Cortes et al. (*173*) in their review of the data on 100 patients treated at Roswell Park Memorial Institute discovered a higher incidence of nonfatal cardiomyopathy (7%), but the rate of fatal cardiomyopathy was similar (2%) to that reported by Gottlieb (*172*). No data exist for correlating schedule dependency and cardiac toxicity, nor are any predictive tests available.

On the basis of the experience with cardiac toxicity, the NCI does not recommend a total dose exceeding 550 mg/m^2. Occasionally, a clinician may be confronted with a question of the significant risk of disease versus the cardiac toxicity of the drug.

TABLE 18.—*Correlation of cardiomyopathy and the total dose of adriamycin*[a]

Total dose, mg/m^2	Patients at risk	Patients with cardiomyopathy		Incidence, %
		Nonfatal	Fatal	
450	663	0	0	0
451–500	23	0	0	0
501–550	31	0	1	3
551–600	14	1	2	21
>600	27	2	6	30
Total	758	3	9	1.6

[a] *See* (*168, 172*).

The clinical judgment of the individual physician should prevail in the decision to exceed the recommended dose.

Bleomycin

Blenoxane (bleomycin sulfate), one of a group of antibiotics originally isolated from *Streptomyces verticellus* by Umezawa et al. (*174*), consists of sulfur-containing polypeptides that separate by paper chromotography into 2 large fractions and 11 subfractions. Bleomycin has been extensively evaluated in the United States under the joint sponsorship of the NCI and Bristol Laboratories Division of Bristol-Myers Company. The drug was recently approved by the Food and Drug Administration for widespread use. Although its exact mechanism of action is unknown, available evidence would indicate that the main mode of action is the inhibition of DNA synthesis with some evidence of lesser inhibition of RNA and protein synthesis.

Bleomycin has palliative value in squamous cell carcinomas of the head and neck region, for which an overall 31% response rate (49/158) is described (*157*). The response ranged from 72% for squamous cell carcinoma of the mouth to 12% for squamous lesions of the tongue. Tumors of the nasopharynx, tonsils, sinuses, and other sites responded in about 30% of cases. The mean duration of response was short, i.e., only 2 months.

Bleomycin also has uniform activity against Hodgkin's disease, reticulum cell sarcoma, and lymphosarcoma. The cumulative response rate in the United States experience (*157*) has been 43% in Hodgkin's disease (29/68), 47% in lymphosarcoma (8/17), and 45% in reticulum cell sarcoma (9/20). Again, the duration of response was short, with a mean of 3.1 and 1.8 months in Hodgkin's disease and in reticulum cell sarcoma, respectively.

Blum et al. (*157*) reported that of 57 patients with testicular carcinoma who could be evaluated and were treated with bleomycin, 37 received the drug as a single agent and 20 in combination with vinblastine. The overall response was 32% for bleomycin alone and 90% for the combination, which is currently being evaluated by several groups for confirmation. Responses were seen in all cell types but, unfortunately, the duration of response was again short for both the single agent (1.5–2 mo) and the combination (2–5 mo) regimen.

The clinical toxicity of bleomycin in over 1,700 cases from worldwide sources (*157*) is outlined in table 19. Bleomycin is noteworthy among antineoplastic drugs for a lack of bone marrow toxicity that makes the drug highly attractive for combination use. Cutaneous reactions, the most common toxicity encountered, occur in about half the patients as mouth ulcers, alopecia, hyperpigmentation, thickening, ulceration, redness, hyperkeratosis, nail changes, rash, vesiculation, tenderness, pruritis, hyperesthesia, peeling, stria, and bleeding. In only 0.2% of treated patients has it been necessary to discontinue bleomycin because of skin reactions.

The most serious toxic effect of bleomycin is pulmonary and its characterization has been extremely difficult. No pathognomonic sign, symptom, X-ray findings, or pathologic changes have been established. The overall incidence is approximately 10% of treated patients. The most frequent manifestation is pneumonitis occasionally progressing to pulmonary fibrosis, which has been fatal in approximately 1% of the patients. Bleomycin-induced pneumonitis apparently produces dyspnea and fine rales, which are in no way different from those caused by infectious pneumonia. An X-ray examination reveals patchy opacities, usually in the lower lung fields.

Several pulmonary function tests in 156 patients receiving bleomycin revealed abnormalities in 20%, consisting of a decrease in total lung volume and vital capacity. No predictive correlation between these changes and the development of pulmonary fibrosis could be ascertained.

The pulmonary effects of toxicity appear to be both dose and age related, and are more common in patients over 70 years of age receiving a total dose of more than 400 mg. In a review of 808 cases (*157*), the data showed definite risk of toxicity in the lungs at about the same rate at *all* dose levels (3–5%) below 450 mg as a total dose; however, there was a significant increase in the overall incidence of toxicity at a total dosage of greater than 450 mg. Similarly, the incidence of toxicity was relatively constant as a function of age up to 70 years (2–6%) and then jumped to 15% (9/61) in patients over 70.

The recommended dose schedule is 10–20 mg/m^2, iv or im, weekly or biweekly, to a total dose of 400 mg. Administration of doses over 400 mg should be approached with great caution; the increased risk of fatal pulmonary toxicity must be recognized.

Acute fulminant reactions resembling anaphylaxis have been observed occasionally in lymphoma patients. Among 808 patients, 4 fulminant deaths and 5 sublethal acute reactions have been reported. Of 149 patients with lymphoma treated with bleomycin, 9 (6%) had this reaction. Based on this find-

TABLE 19.—*Common manifestations of bleomycin toxicity (in percent) reported by various groups*

Manifestations of toxicity	United States	Japan	EORTC [a]	Scandinavia
Mucositis	22	—	≈20	38
Alopecia	13	29	≈30	41
Pigmentation	8	20	—	16 [b]
Pyrexia	26	36	≈50 (iv) ≈15 (im)	20
Anorexia	17	32	—	—
Nausea	14	42 [c]	≈5	—
Vomiting	10			15 [b]
Pulmonary effects	10	9 [d]	≈3	12
Total number of cases for evaluation [e]	808	540	237	154

[a] European Organization for Research and Treatment of Cancer.
[b] In 85 patients.
[c] In 72 patients.
[d] In 468 patients.
[e] *Note* number of cases, not percentage.

ing, it is recommended that patients with lymphoma receive a test dose of bleomycin, e.g., 1 mg followed by a 24-hour observation period. If there are no outward effects, then full dosage can be administered.

The current indications for bleomycin as a single antineoplastic agent are limited to end-stage patients resistant to conventional therapy, or to those who are unable to receive conventional therapy because of bone marrow depression. The relative efficacy of bleomycin compared with known drugs is limited by the current lack of comparative controlled trials. At present, the only comparisons that can be made to other therapies are on the basis of response rates in often noncomparable patient populations. Based on this rationale, bleomycin may be expected to provide benefit to patients with lymphoma, testicular tumors, and squamous cell carcinoma in certain anatomic sites that are refractory to conventional therapy or when further conventional treatment is prevented by bone marrow toxicity.

The greatest potential for bleomycin may lie in combination chemotherapy because of its lack of marrow toxicity and its characteristic induction of early responses of short duration. The clinical trials in progress with bleomycin in combination therapy in a number of tumor types may ultimately define the role of this drug.

1,2-Di(3,5-dioxopiperazine-1-yl)propane

This compound, known as ICRF 159, was developed by scientists at the Imperial Cancer Research Fund facilities in London, England (*175*). Although the exact mechanism of action has not been fully elucidated, the cytotoxic effect occurs during late prophase and early metaphase (G_2–M) and involves inhibition of DNA synthesis (*176, 177*).

ICRF 159 was selected for clinical trials on the basis of antitumor activity in L1210 leukemia and in the LLT. The drug exhibits definite schedule dependency in L1210, in which it is active on an intermittent schedule and less so when administered daily. The most exciting data were observed in the LLT which, when implanted in the flank of a mouse, metastasizes spontaneously to the lungs. Salsbury et al. (*178*) examined this property of the tumor in a series of experiments comparing the ability of ICRF 159 and cyclophosphamide to prevent metastases. ICRF 159 completely inhibited metastases formation at doses that had little influence on the rate of growth of the primary tumor implant. Inhibition was produced by the effect of ICRF 159 on the development of blood vessels in the invading margins of the primary tumor. Cyclophosphamide did not prevent metastatic spread when used on schedules similar to ICRF 159 but did not decrease the number and size of the metastases. All the untreated control mice developed metastases.

Initial clinical trials in Great Britain were performed on a schedule of daily oral doses of 20–30 mg/kg/day in divided doses administered until the occurrence of hematologic toxicity (*179–182*). Leukopenia and thrombocytopenia occur within a few days of treatment initiation and are dose related (5 g total dose is the current British restriction). Dramatic decreases in the number of circulating blast cells are described in leukemia patients and no cross-resistance to their antileukemia drugs has been noted.

Two studies with ICRF 159 were completed in the United States (*183, 184*). Based on the pharmacokinetic study in man, the recommended oral dose for phase II clinical studies is 3 g/m^2 given once weekly. Phase II studies in the major signal tumors are in progress.

Chromomycin A3

This anticancer antibiotic of Japanese origin was isolated from a culture of *Streptomyces griseus* No. 7 (*185*) and is commercially available in Japan as Toyomycin. Chromomycin A3 is an aureolic acid analogue consisting of an aglycone moiety (chromomycinone) and five attached pentoses (*186–188*). Studies of the mechanism of action show that chromomycin A3, in the presence of Mg^{2+}, inhibits DNA-dependent RNA polymerase (*189–191*). The interaction of the drug with DNA requires the presence of a guanine base (*189, 190, 192*). Inhibition of DNA polymerase has been demonstrated by Hartmann et al. (*193*).

The drug was selected for clinical trial on the basis of its activity against P388 leukemia in mice, in which it shows superiority to both mithramycin and olivomycin. Cytostatic activity is also reported in a number of experimental animal tumors including Yoshida's sarcoma, sarcoma 180, Ehrlich's ascites tumor, and others (*194–196*). Little activity was found against L1210 leukemia in mice.

Preclinical pharmacokinetic studies show that the drug is rapidly excreted in bile and urine, and almost totally cleared from the plasma in 3 hours after administration (*197*). Chromomycin A3 has been used as a single agent in more than 500 Japanese and South African patients with a wide variety of neoplastic diseases (*198, 199*). Objective responses are reported in malignant lymphomas (*200–204*) and in solid tumors including bronchogenic and gastrointestinal adenocarcinomas, carcinomas of the female genital tract, malignant gliomas, and soft-tissue sarcomas (*202, 203, 205*). The drug has also been used in combination with radiotherapy (*203, 206*) and with alkylating agents; a synergistic effect is noted (*207, 208*).

Toxicity reported in Japanese and South African studies is surprisingly low, consisting of nausea and vomiting after daily doses of 1 mg or higher (*202, 203*), moderate leukopenia (*200, 202, 203*), and local reaction at the injection site with necrosis after extravasal administration. Recent phase I studies in the United States reveal that renal toxicity is dose limiting when a daily iv dose for 5 days is escalated above 0.9 mg/m^2. Hypocalcemia, which has not been detected in previous studies, is also reported. Other dose schedules are now being explored, and phase II studies with lower doses are being proposed to avoid the dose-limiting side effects.

N-(2-chloroethyl)-*N'*-(2-chloroethyl)-*N'*-*O*-propylene phosphate diamide

This analogue of cyclophosphamide, which is known as iphosphamide (NSC-109724), was investigated because of superior cytostatic activity in the leukemia L1210 system. Table 20 compares the L1210 activity of iphosphamide with that of cyclophosphamide and another analogue (NSC-109723). Besides the superiority to cyclophosphamide in L1210, activity for iphosphamide has also been noted in Lewis lung, Ehrlich ascites, and Yoshida sarcoma systems.

The initial investigation of iphosphamide was sponsored by the Asta-Werke Chemical Company of Bielefeld, West Germany (*209–214*), and extensive trials have been made by German investigators. Iphosphamide appears to have many characteristics in common with cyclophosphamide, i.e., toxicities (dose-related, reversible leukopenia with relative platelet sparing, nausea, and cystitis), forms of administration (iv or oral), and range of tumor activity in man. Preliminary German studies with single large-dose therapy (100–150 mg/kg) have

TABLE 20.—*Relative effectiveness against L1210 ascites*

Treatment			Cytoxan (NSC-26271)	Trophosphamide (NSC-109723)	Iphosphamide (NSC-109724)
Route	Schedule		ILS % (OD)[a]	ILS % (OD)[a]	ILS % (OD)[a]
	Frequency	Day(s)			
ip	Once	1	300 (180)	300 (500)	300 (300)
ip	Every 3 hr	1	150 (300/8)	300 (300/8)	120 (300/8)
ip	Daily	1–9	50 (39)	95 (39)	50 (65)
ip	Daily	1, 5, 9	70 (108)	135 (65)	85 (39)
ip	Every 3 hr	1, 5, 9	60 (108/8)	145 (180/8)	110 (180/8)
ip	Daily	1, 9	120 (108)	300 (300)	125 (180)
ip	Every 3 hr	1, 9	70 (180/8)	100 (180/8)	155 (300/8)
oral	Once	1	153 (300)	300 (180)	300 (500)
oral	Daily	1, 9	100 (108)	300 (500)	300 (500)

[a] ILS % = percentage increase in survival time over controls; OD = optimal dose (mg/kg/day).

TABLE 21.—*Phase I studies with iphosphamide*

Study[a]	Number of patients evaluated	Dose schedule	Dose, mg/m²			Incidence of toxicity				
			Start	Highest escalation	Maximum tolerated	Gastro-intestinal	Hematologic	Hepatic	Hematuria	CNS
Cohen	39	Single iv	200	5,000	5,000	41.3	15.3	—	25.6	5.1

[a] Pharmacokinetic studies included.

TABLE 22.—*Phase II studies with iphosphamide*

Carcinoma	Number of patients evaluated	Number of responses		Response, %	Investigators
		CR	CR + PR		
Breast	20[a]	—	4	20	Ahman, 1973
Colon	33[b]	—	1	3	Kovach, 1973

[a] 4,000 mg/m² iv every 4 wk.
[b] 4,000 mg/m² iv every 3 wk.

yielded reports of high activity in oat cell tumors of the lung, ovarian cancer, breast cancer, and lymphomas.

A phase I study (table 21) by Drs. Cohen and Selawry in the NCI–VA Medical Oncology Service reached a maximum tolerated single dose (iv) of 5,000 mg/m². The dose recommended for phase II trial ranges between 3,800 and 5,000 mg/m². The pharmacokinetic studies revealed peak urinary alkylating activity continuing for 3 days.

Phase II results presently available in two signal tumors, adenocarcinoma of the breast and the colon, do not suggest superiority of this analogue over cyclophosphamide (table 22). There is an apparent need for controlled randomized phase II studies comparing the effect of both drugs in all signal tumors. However, iphosphamide is not too appealing since, in addition to the toxicities it has in common with cyclophosphamide, it produces more severe hematuria (caused by hemorrhagic cystitis).

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APPENDIX I

The Linear Array

STAGE I — SELECTION AND ACQUISITION OF AGENTS FOR SCREENING

STEP 1	STEP 2	STEP 3	STEP 4	STEP 5
ACQUIRE DATA ON POTENTIAL AGENTS FOR SCREENING	SELECT AGENTS TO BE ACQUIRED FOR SCREENING, ELIMINATING DUPLICATES WHERE POSSIBLE: CRITERIA FOR SELECTION 1. ANTI-TUMOR STRUCTURE ACTIVITY RELATIONSHIPS A. EMPHASIZE NEW AND PROMISING STRUCTURAL CLASSES B. DE-EMPHASIZE ADEQUATELY STUDIED CLASSES OR NON-PRODUCTIVE STRUCTURAL CLASSES 2. NEW STRUCTURES OR ANALOGUES DEVELOPED THROUGH CHEMICAL, BIOCHEMICAL, CELL-CYCLE AND KINETIC, PHARMACOLOGIC AND PHARMACEUTICAL RATIONALES 3. BIOLOGICAL ACTIVITY DEMONSTRATED IN OTHER SYSTEMS AND PROGRAMS (non-NCI) 4. CURRENT AND POTENTIAL SOURCES FOR NATURAL PRODUCTS 5. NATURE AND EXTENT OF RANDOM SYNTHETIC COMPOUND INVENTORY AND INPUT 6. SUBMISSIONS OF AGENTS TO DR&D FOR SCREENING WITHOUT SPECIFIC SOLICITATION	ACQUIRE AND RECEIVE AGENTS FOR SCREENING SPECIFYING (WHERE POSSIBLE): A. QUANTITY DESIRED B. PHYSICAL CONSTANTS C. CHEMICAL STRUCTURE D. SUPPORTING INFORMATION; LOT NUMBER SOLUBILITY, STABILITY AND BIOLOGICAL ACTIVITY	REVIEW IDENTIFYING DATA AND CLASSIFY THE AGENT CHEMICALLY AND BIOLOGICALLY; IDENTIFY AND ELIMINATE DUPLICATE AGENTS.	ASSIGN NSC NUMBER, INVENTORY AND STORE AGENTS

STAGE IIA — DETERMINATION OF ANTI-TUMOR ACTIVITY OF NEW AGENTS

STEP 1	STEP 2	STEP 3	STEP 4	STEP 5	STEP 6
ESTABLISH CRITERIA TO BE UTILIZED IN ASSIGNING TESTING REQUIREMENTS FOR VARIOUS CLASSES OF AGENTS; APPLY CRITERIA IN DEFINING TEST SERIES FOR SPECIFIC AGENTS.	ASSIGN A PRIORITY TO AGENTS FOR TESTING: PRIORITY 1. UNSTABLE MATERIALS PRIORITY 2. MATERIALS DEMONSTRATING CLINICAL ACTIVITY IN MAN; USED BY INVESTIGATORS OUTSIDE OF NCI PRIORITY 3. MATERIALS DEMONSTRATING CHARACTERISTICS SUPERIOR TO THOSE PREVIOUSLY TESTED BY NCI, AND ADVANCED BY INVESTIGATORS OUTSIDE OF NCI PRIORITY 4. MATERIALS DEVELOPED OR ACQUIRED AT DR&D REQUEST PRIORITY 5. OTHERS TO BE TESTED IN ORDER OF RECEIPT	ASSIGN AND DISTRIBUTE AGENTS TO TESTING LABORATORIES	DETERMINE ACTIVITY IN CURRENT ROUTINE TEST SYSTEMS L1210 SINGLE TREATMENT L1210 DAILY OTHER TEST SYSTEMS SPECIAL PURPOSE TESTING FOLLOWING NON-ROUTINE PROTOCOLS	DATA PROCESSING ADDITIONAL TESTING REQUIRED FOR EVALUATION	EVALUATE SCREENING DATA. DECIDE UPON ACTIVE AGENTS TO BE RECOMMENDED FOR CONSIDERATION IN STAGE II DECISION NETWORK; RECYCLE WHEN DATA IS INADEQUATE TO ALLOW PROPER EVALUATION OF ACTIVITY
STEP 1a DEVELOP AND EVALUATE NEW TEST SYSTEMS					

RECYCLE SYNTHETICS

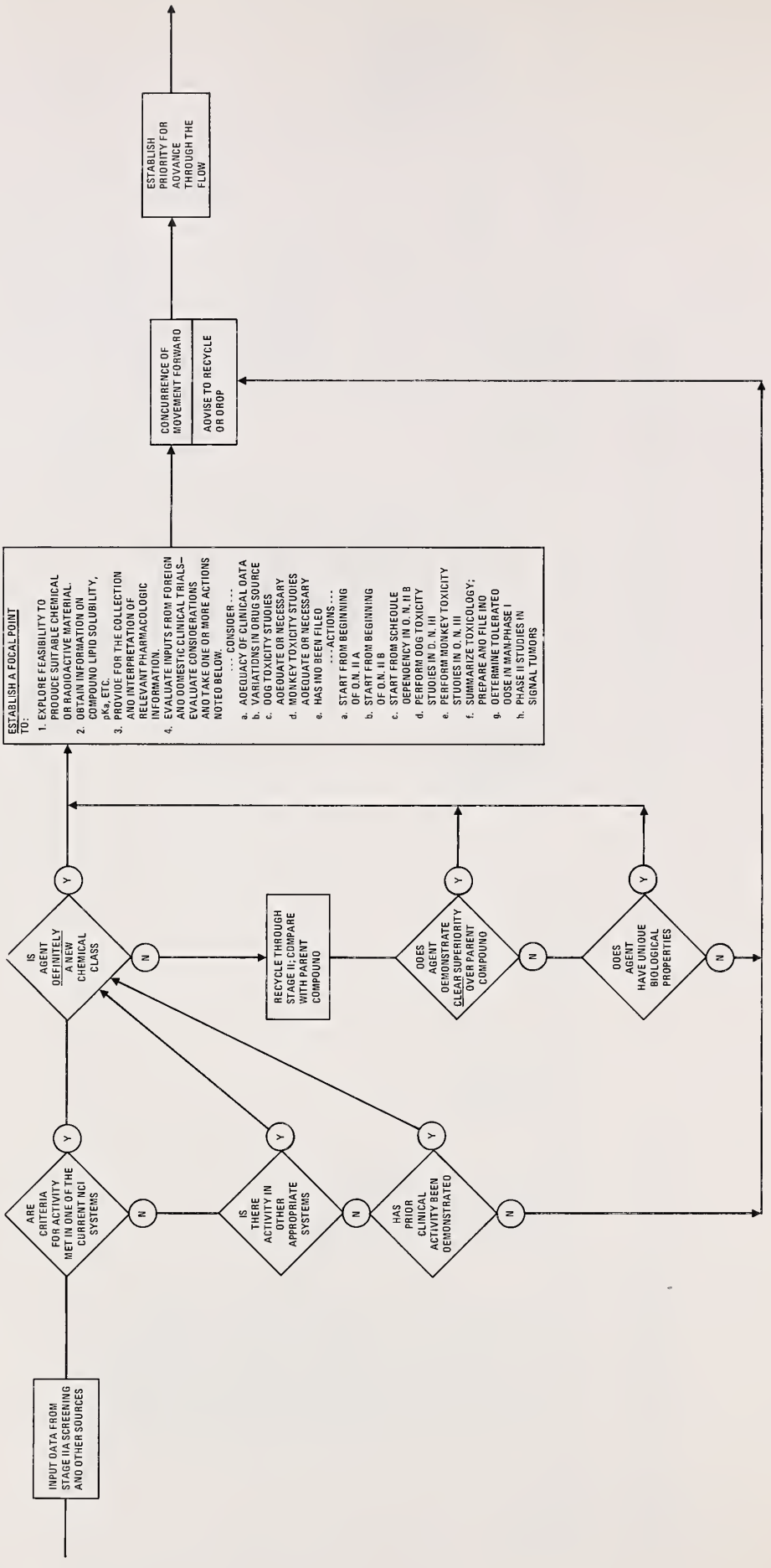
RECYCLE NATURAL PRODUCT FRACTIONS DURING PURIFICATION EFFORTS

REQUESTS FROM STAGE II DECISION NETWORK

RECYCLE FINAL PURIFIED FRACTION

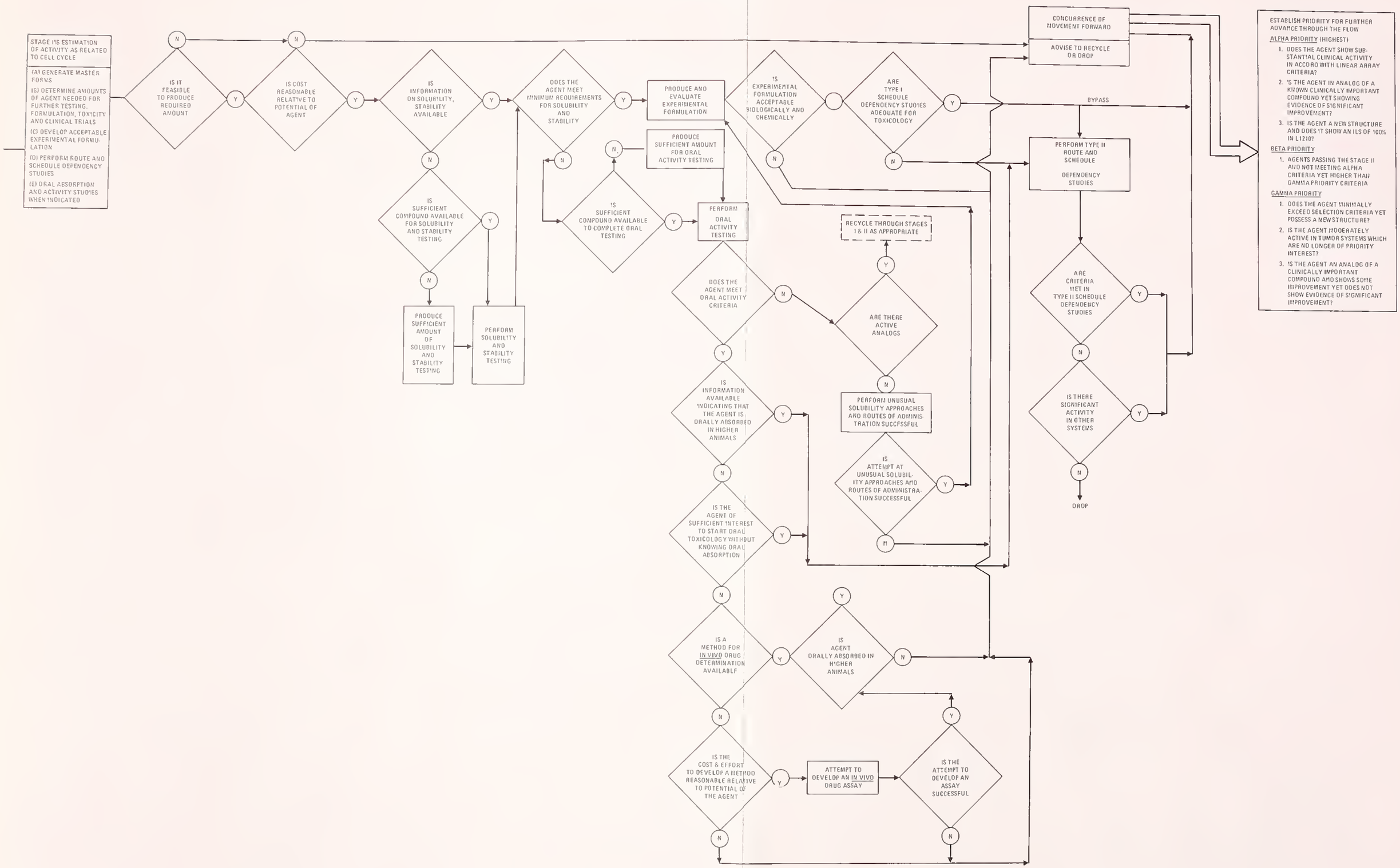
DECISION NETWORK IIA

SELECTION OF AGENTS FOR PROGRAMMED ACTIVITY IN MAN



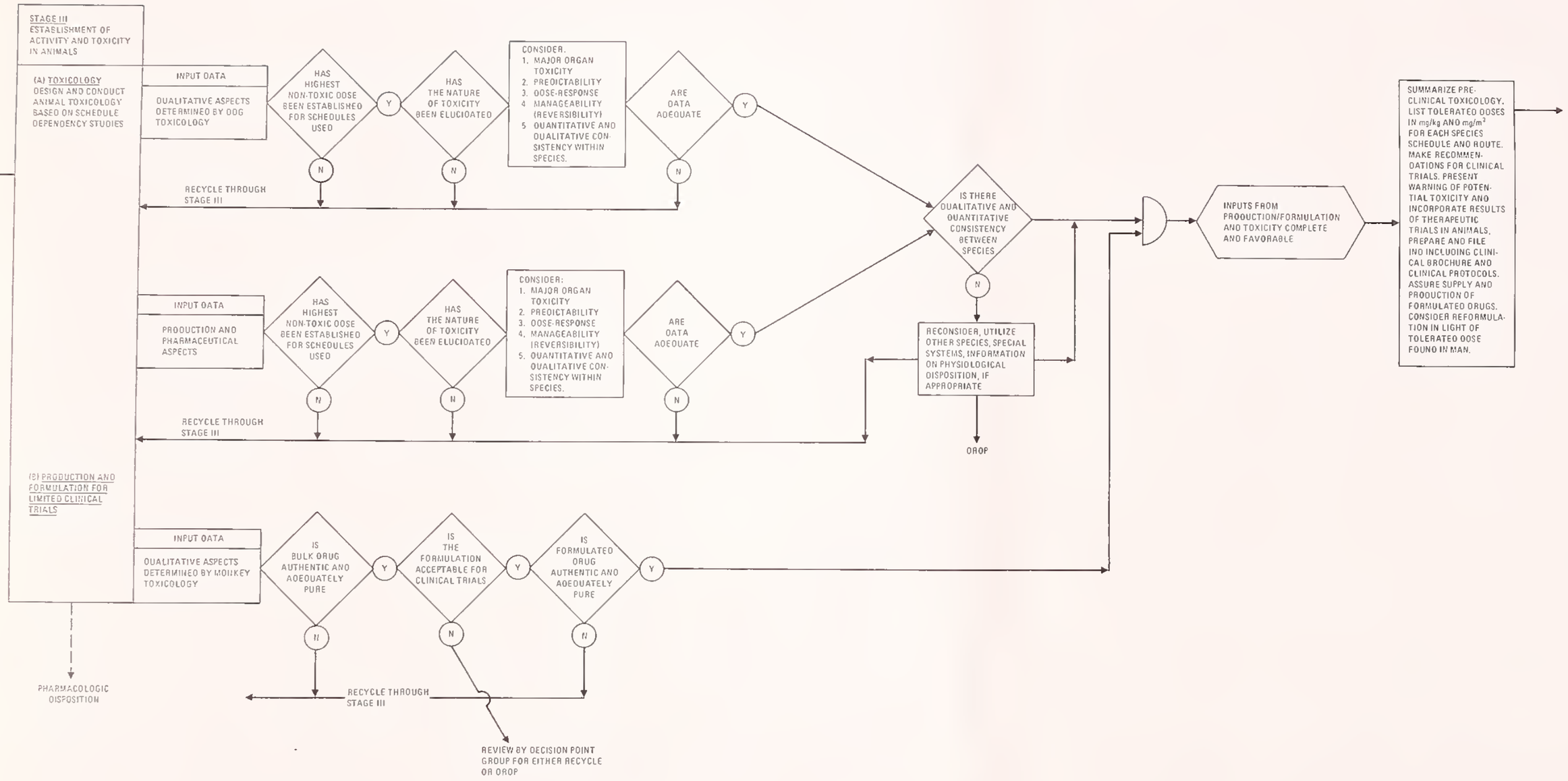
DECISION NETWORK IIB

SELECTION OF AGENTS FOR PROGRAMMED ACTIVITY IN MAN



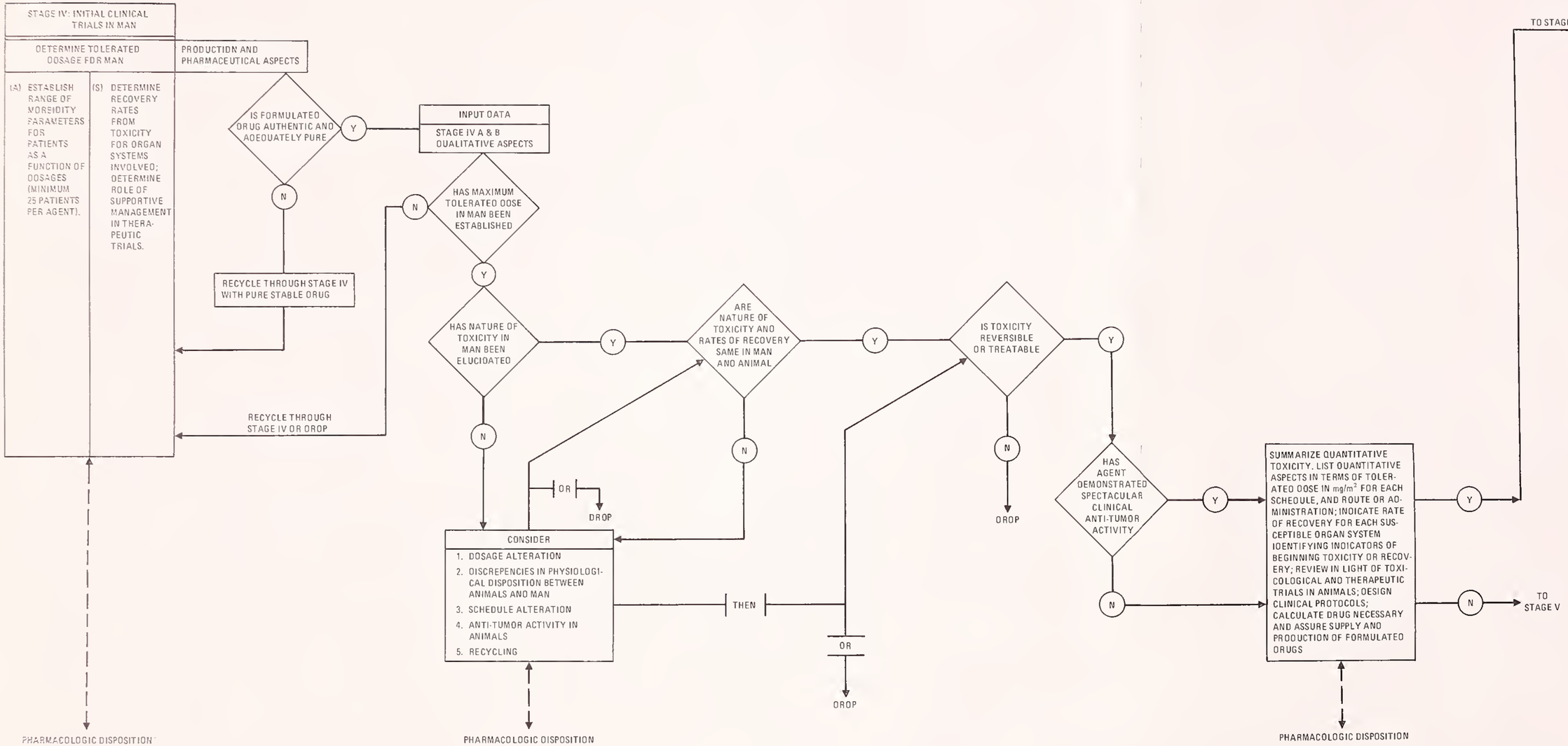
DECISION NETWORK III

SELECTION OF AGENTS FOR INITIAL CLINICAL TRIALS IN MAN



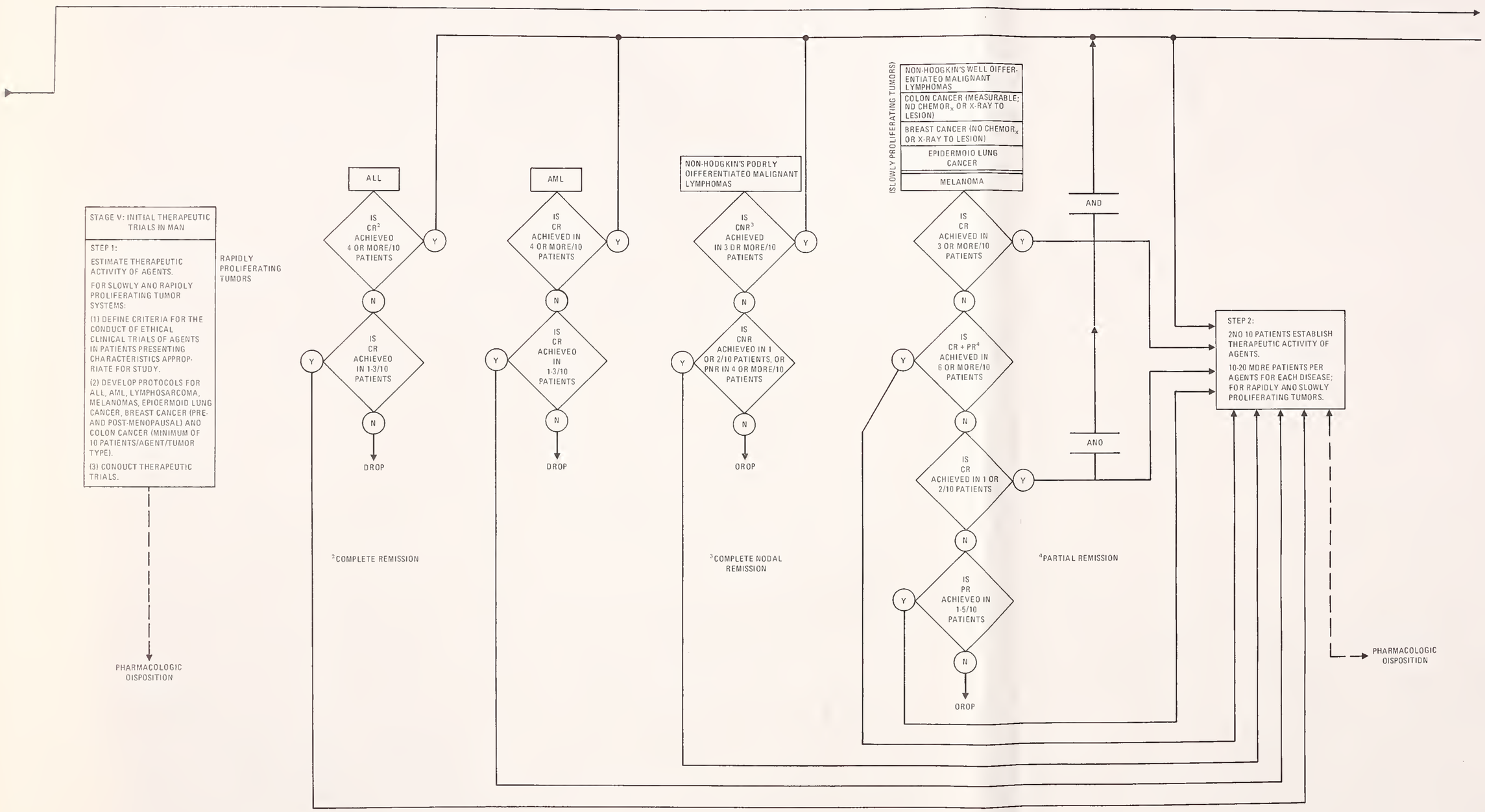
DECISION NETWORK IV

SELECTION OF AGENTS FOR THERAPEUTIC TRIALS



DECISION NETWORK V

SELECTION OF AGENTS FOR INITIAL ERADICATIVE TRIALS IN MAN



DECISION NETWORK VI

SELECTION OF AGENTS FOR INITIAL ERADICATIVE TRIALS IN MAN

ACUTE LYMPHOCYTIC LEUKEMIA[5]

AGENT PRODUCES COMPLETE REMISSION (CR) IN: 1 OR MORE/20 PATIENTS IN SECOND PASS

IS CR ACHIEVED IN 8 OR MORE/20 PATIENTS — Y

N

IS COMPLETE REMISSION ACHIEVED IN 2-7/20 PATIENTS — N → DROP

Y

IS CR ACHIEVED WITH TRIVIAL TOXICITY TO HOST &/OR IS BONE MARROW-GI TRACT SPARED — Y

N

IS AGENT SUITABLE FOR COMBINATION R_x — Y

N

IS AGENT IN NEW CHEMICAL CLASS — Y → TO STAGE VI AND/OR RECYCLE THROUGH S.I OR DROP

N

IS AGENT NON-CELL CYCLE SPECIFIC — Y → TO STAGE VI AND/OR RECYCLE THROUGH S.I OR DROP

N

IS AGENT HIGHLY DIFFUSIBLE — Y → CONSIDER FOR SPECIAL USES

N → DROP

[5]SIMILAR NETWORKS FOR OTHER TUMORS WITH APPROPRIATE NUMERICAL DATA.

STAGE VI: INITIAL ERADICATIVE TIRALS IN MAN

STEP 1: TYPE III TOXICOLOGY

DESIGN AND CONDUCT TOXICOLOGIC AND PHARMACOLOGIC STUDIES IN ANIMAL SYSTEMS TO DETERMINE SAFETY OF THE POSSIBLE INDICATED OOSAGES OF AGENTS OR COMBINATIONS OF OTHER TREATMENT PRIOR TO MORE EXTENSIVE CHRONIC STUDIES IN MAN.

STEP 2: APPROXIMATE EXTENT OF TUMOR ERAOICATION CAPACITY OF AGENTS

DEVELOP PROTOCOLS FOR EXTENT OF TUMOR ERADICATION IN PATIENTS WITH RESPONSIVE TUMOR USING AGENT SINGLY AND IN COMBINATION.

PHARMACOLOGIC DISPOSITION

MONITORING POINT: VALIDATE THERAPEUTIC INDEX DATA FROM ANIMALS IN RELATION TO THERAPEUTIC INDEX FOR MAN.

1. ANALYZE OATA ON C x T (mg/m^2) EXPOSURE FOR TUMOR CELL KILL IN ANIMAL MODEL SYSTEMS ANO PATIENTS.
2. ANALYZE DATA ON C x T (mg/m^2) EXPOSURES FOR MORBIDITY IN ANIMAL MOOEL SYSTEMS AND PATIENTS.
3. DETERMINE ACCURACY OF PREDICTABILITY OF MODEL SYSTEMS FOR THERAPEUTIC INDEX IN MAN.
4. ADJUST MODEL SYSTEMS AS REQUIREO THROUGH FEEOBACK.
5. APPRAISE STRUCTURE-FUNCTION RELATIONSHIPS.

DECISION POINT

STAGE 6 SELECTION OF AGENTS FOR BROAO CLINICAL TRIALS:
DISCUSSION BETWEEN NCI STAFF ANO:

1. DISEASE ORIENTEO TASK FORCES
2. COOPERATIVE CLINICAL GROUPS
3. INOEPENDENT INVESTIGATORS AND INSTITUTIONS

STAGE VII: DETERMINATION OF ROLE OF AGENTS IN TOTAL CARE OF THE PATIENT

1. INSURE AVAILABILITY OF NEW EFFECTIVE AGENTS.
2. INSURE DISSEMINATION OF INFORMATION ON NEW EFFECTIVE AGENTS.
3. DEVELOP AND UTILIZE METHODS FDR COLLECTION AND ANALYSIS OF PERTINENT DATA.
4. BROAD TRIAL BY SPECIALISTS AND COOPERATIVE GROUPS.

PHARMACOLOGIC DISPOSITION

INTRODUCTION TO GENERAL MEDICAL PRACTICE

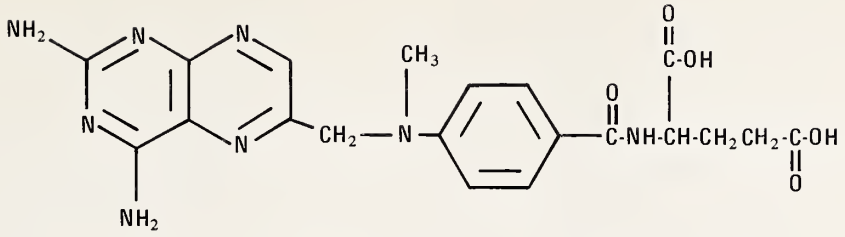
APPENDIX II

Chemical Structures of

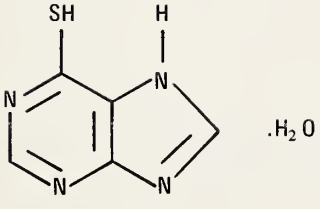
Current Interest to the

National Cancer Institute,

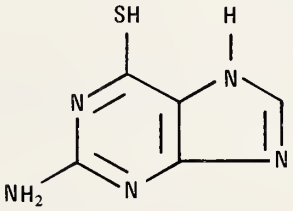
Division of Cancer Treatment



Methotrexate
NSC-740



6-Mercaptopurine
NSC-755



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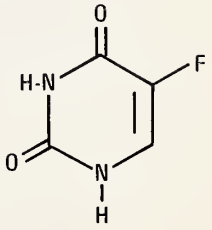
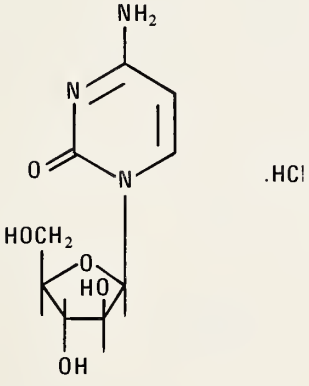
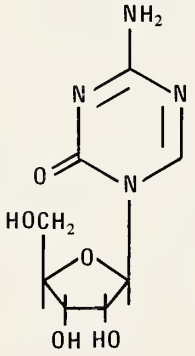


5-Fluorouracil
NSC-19893



Cytosine Arabinoside
NSC-63878



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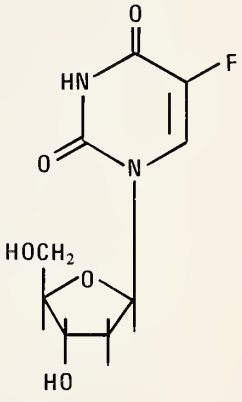
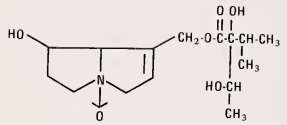
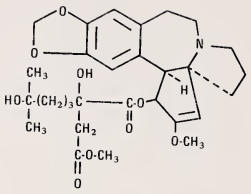


5-FUDR
NSC-27640



Indicine, N-oxide
NSC-132319



Homoharringtonine
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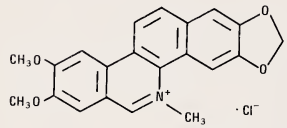

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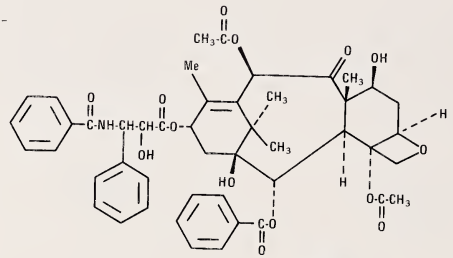

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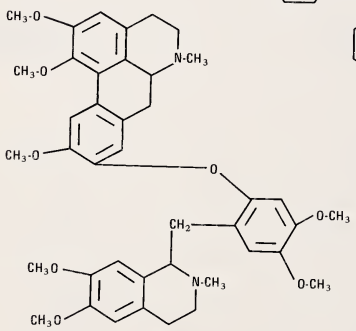
NSC-125973



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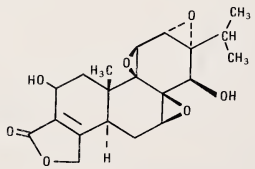

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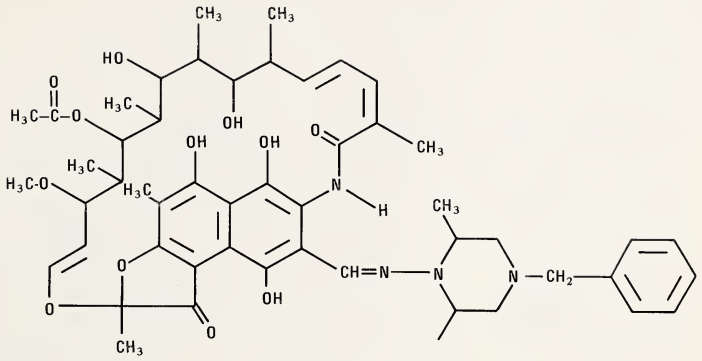

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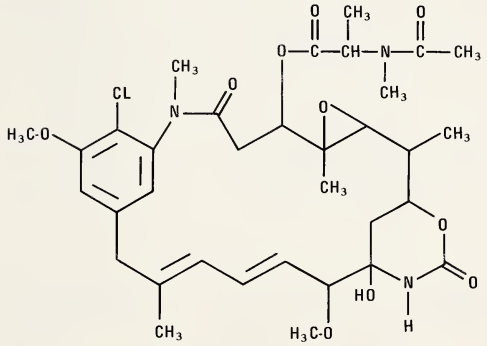

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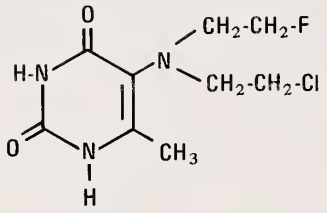

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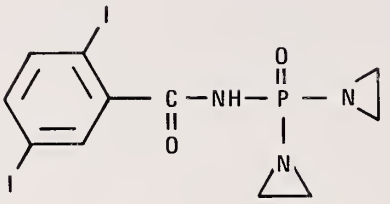

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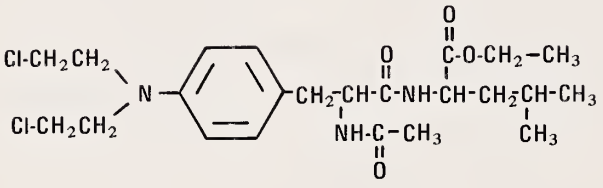

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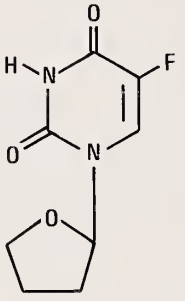

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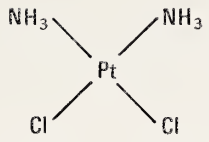

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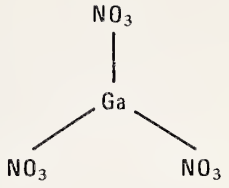

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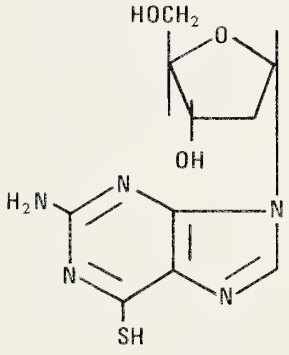

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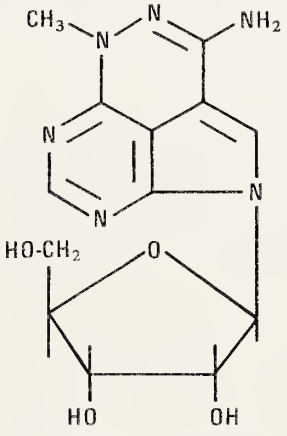

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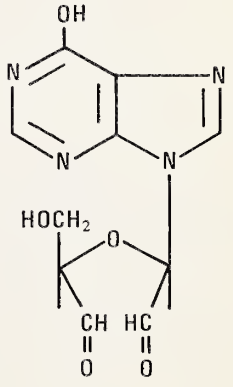

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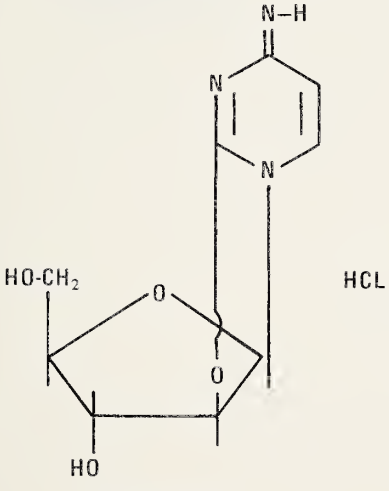

HCL

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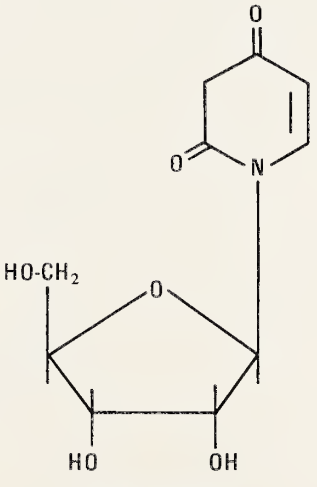

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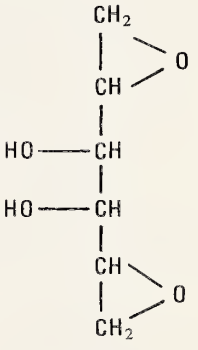

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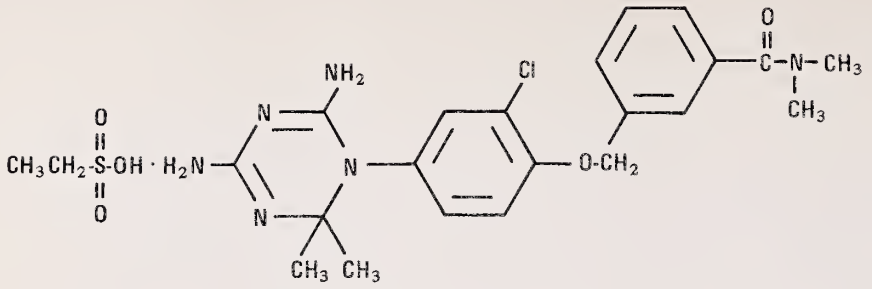

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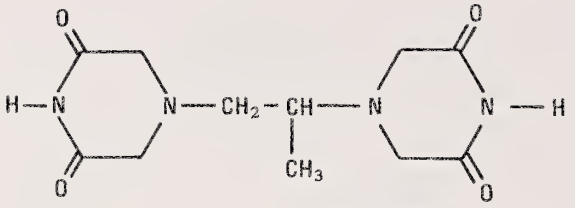
NSC-139105



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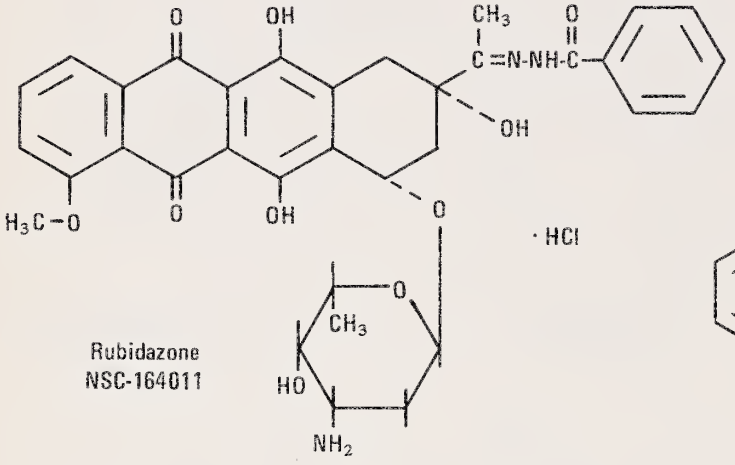

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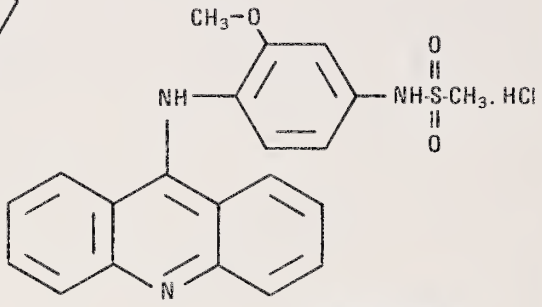

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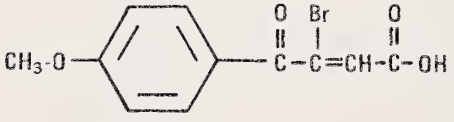
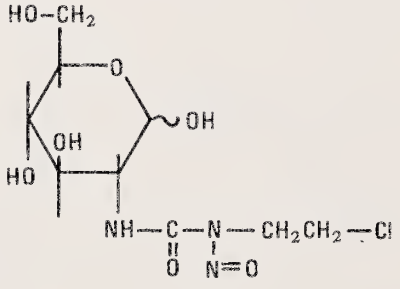
NSC-141549



Cytembena
NSC-104801



Chlorozotocin
NSC-178248

APPENDIX III

A. PROTOCOLS FOR IN VIVO SCREENING SYSTEMS

Lymphoid Leukemia L1210

Ascitic fluid implanted in BDF_1 or CDF_1 mice. Treatment begins 24 hours after implants. Results expressed as a percentage of control survival time. Under normal conditions, the inoculum site for primary screening is ip, the drug is administered ip, and the parameter is mean survival time. For testing other than primary screening, the information in this Protocol may vary by instruction from Drug Research and Development (DR&D). Origin of tumor line: induced in 1948 in spleen and lymph nodes of mice by painting skin with 3-methylcholanthrene (*1*).

Animals

Propagation: DBA/2 mice (or BDF_1 or CDF_1 for one generation if DBA/2 are not available).
Testing: BDF_1 (C57BL/6 × DBA/2) or CDF_1 (BALB/c × DBA/2) mice.
Weight: Within a 3-g weight range, with a minimum weight of 18 g for males and 17 g for females.
Sex: One sex used for all test and control animals in one experiment.

Experiment size

General testing: Six animals per test group.
Control groups: Number of animals varies according to number of test groups.

Tumor transfer

Implant: Inject ip.
Size of implant: 0.1 ml of diluted ascitic fluid containing 10^5 cells.
Time of transfer for propagation: day 6 or 7.
Time of transfer for testing: day 6 or 7.

Testing schedule

Day 0: Implant tumor. Run bacterial cultures. Determine solubilities. Thaw solutions. Prepare materials. Run positive control in every odd-numbered experiment. Record survivors daily.
Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals. Treat as instructed.
Day 2: Recheck cultures. Discontinue testing if contaminated.
Day 5: Weigh animals and record. Prepare fresh compound for subsequent testing.
Day 20: If there are no survivors except those treated with positive control compound, evaluate experiment.
Day 30: Kill all survivors and evaluate experiment.

Quality control

Acceptable control survival time is 8–11 days. Positive control compound is 5-fluorouracil (NSC-19893): single dose = 200 mg/kg/injection, intermittent dose = 60 mg/kg/injection, and chronic dose = 20 mg/kg/injection. Treated/control (T/C) lower limit for positive control compound is ≥135%. Check control deaths, no takes, etc.

Evaluation

Compute mean animal weight on days 1 and 5, and at the completion of testing compute T/C for all test groups with > 65% survivors on day 5. A T/C value ≤85% indicates a toxic test. An initial T/C ≥125% is considered necessary to demonstrate activity. A reproduced T/C ≥125% is considered worthy of further study. For confirmed activity, a synthetic must have two multidose assays (each performed at a different laboratory) that produce a T/C ≥125%; a natural product must have two different samples that produce a T/C ≥125% in multidose assays.

Reporting

On the final day of testing, prepare final control and test reports and send them for key-punching.

Lymphocytic Leukemia P388

Ascitic fluid implanted in BDF_1 or CDF_1 mice. Treatment begins 24 hours after implant. Results expressed as a percentage of control survival time. Under normal conditions, the inoculum site for primary screening is ip, the drug is administered ip daily for 9 days, and the parameter is median survival time. For testing other than primary screening, the information in this Protocol may vary by instruction from DR&D. Origin of tumor line: induced in 1955 in a DBA/2 mouse by painting with 3-methylcholanthrene (*2*).

Animals

Propagation: DBA/2 mice (or BDF_1 or CDF_1 for one generation if DBA/2 are not available).
Testing: BDF_1 (C57BL/6 × DBA/2) or CDF_1 (BALB/c × DBA/2) mice.
Weight: Within a 3-g weight range, with a minimum weight of 18 g for males and 17 g for females.
Sex: One sex used for all test and control animals in one experiment.

Experiment size

General testing: Six animals per test group.
Control groups: Number of animals varies according to number of test groups.

Tumor transfer

Implant: Inject ip.
Size of implant: 0.1 ml diluted ascitic fluid containing 10^6 cells.
Time of transfer for propagation: day 7.
Time of transfer for testing: day 6 or 7.

Testing schedule

Day 0: Implant tumor. Run bacterial cultures. Determine solubilities. Thaw solutions. Prepare materials. Run positive control in every odd-numbered experiment. Record survivors daily.
Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals. Treat as instructed.
Day 2: Recheck cultures. Discontinue testing if contaminated.
Day 5: Weigh animals and record. Prepare fresh compound for subsequent testing.
Day 20: If there are no survivors except those treated with positive control compound, evaluate experiment.
Day 30: Kill all survivors and evaluate experiment.

Quality control

Acceptable median survival time is 9–14 days. Positive control compound is 5-fluorouracil (NSC-19893): single dose = 200 mg/kg/injection, intermittent dose = 60 mg/kg/injection, and chronic dose = 20 mg/kg/injection. T/C lower limit for positive control compound is ≥135%. Check control deaths, no-takes, etc.

Evaluation

Compute mean animal weight on days 1 and 5, and at the completion of testing compute T/C for all test groups with < 65% survivors on day 5. A T/C value ≤ 85% indicates a toxic test. An initial T/C ≥125% is considered necessary to demonstrate activity. A reproduced T/C ≥125% is considered worthy of further study. For confirmed activity, a synthetic must have two multidose assays (each performed at a different laboratory) that produce a T/C ≥125%; a natural product must have two different samples that produce a T/C ≥ 125% in multidose assays.

Reporting

On the final day of testing, prepare final control and test reports and send them for key-punching.

Melanotic Melanoma B16

Tumor homogenate implanted ip or sc in BDF_1 mice. Treatment begins 24 hours after either ip or sc implant or is delayed until a subcutaneous tumor of specified size (usually approximately 400 mg) can be palpated. Results expressed as a percentage of control survival time. The drug is administered ip daily for 9 days and the parameter is median survival time. However, the procedures in this Protocol may vary by instruction from DR&D. Origin of tumor line: arose spontaneously in 1954 at the base of the ear in a C57BL/6 mouse (*3*).

Animals

Propagation: C57BL/6 mice.
Testing: BDF_1 (C57BL/6 × DBA/2) mice.
Weight: Within a 3-g weight range, with a minimum weight of 18 g for males and 17 g for females.
Sex: One sex used for all test and control animals in one experiment.

Experiment size

General testing: Ten animals per test group.
Control groups: Number of animals varies according to number of test groups.

Tumor transfer

Propagation: Implant fragment sc by trocar or 12-gauge needle or tumor homogenate (*see* below) every 10–14 days into axillary region with puncture in inguinal region.
Testing: Excise subcutaneous tumor on day 10–14.
Homogenate: Mix 1 g of tumor with 10 ml of cold balanced salt solution and homogenize, and implant 0.5 ml of this tumor homogenate ip or sc.
Fragment: A 25-mg fragment may be implanted sc.

Testing schedule

Day 0: Implant tumor. Run bacterial cultures. Determine solubilities. Thaw solutions. Prepare materials. Run positive control in every odd-numbered experiment. Record survivors daily.
Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals. Treat as instructed.
Day 2: Recheck cultures. Discontinue testing if contaminated.
Day 5: Weigh animals and record. Prepare fresh compound for subsequent testing.
Day 60: Kill all survivors and evaluate experiment.

Quality control

Acceptable median control survival time is 14–22 days for intraperitoneal tumor. Positive control compound is 5-fluorouracil (NSC-19893) (chronic dose = 20 mg/kg/injection) or cyclophosphamide (NSC-26271) (chronic dose = 50 mg/kg/injection). T/C lower limit for positive control compound is ≥135%. Check control deaths, no-takes, etc.

Evaluation

Compute mean animal weight on days 1 and 5, and at the completion of testing compute T/C for all test groups with > 65% survivors on day 5. A T/C value ≤85% indicates a toxic test. An initial T/C ≥ 125% is considered necessary to demonstrate activity. A reproduced T/C ≥ 125% is considered worthy of further study. For confirmed activity, a synthetic must have two multidose assays (each performed at a different laboratory) that produce a T/C ≥125%; a natural product must have two different samples that produce a T/C ≥125% in multidose assays.

Reporting

On the final day of testing, prepare final control and test reports and send them for key-punching.

Lewis Lung Carcinoma

Tumor may be implanted sc as a 2- to 4-mm fragment or im as a 2×10^6 cell inoculum. Treatment begins 24 hours after implant or is delayed until a tumor of specified size (usually approximately 400 mg) can be palpated. The drug is administered ip daily for 11 days, and the results are expressed as a percentage of the control. Procedures in this Protocol may vary by instruction from DR&D. Origin of tumor: arose spontaneously in 1951 as carcinoma of the lung in a C57BL/6 mouse [Lewis, MR: Unpublished data; (*4*)].

Animals

Propagation: C57BL/6 mice.
Testing: BDF_1 mice.
Weight: Within a 3-g weight range, with a minimum weight range of 18 g for males and 17 g for females.
Sex: One sex used for all test and control animals in one experiment.

Experiment size

General testing: Six animals per test group for sc implant, or 10 for im implant.
Control groups: Number of animals varies according to number of test groups.

Tumor transfer

Implant: Inject cells im in hind leg or implant fragment sc in axillary region with puncture in inguinal region.
Time of transfer for propagation: days 12–14.
Time of transfer for testing: days 12–14.

Testing schedule

Day 0: Implant tumor. Run bacterial cultures. Determine solubilities. Thaw solutions. Prepare materials. Run positive control in every odd-numbered experiment. Record survivors daily.
Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals. Begin treatment as instructed.

Day 2: Recheck cultures. Discontinue testing if contaminated.
Day 5: Weigh animals and record (if applicable). Prepare fresh compound for subsequent testing.
Final day: Kill all survivors and evaluate experiment.

Quality control

Acceptable im tumor weight on day 12 is 500–2,500 mg. Acceptable im tumor median survival time is 18–28 days. Positive control compound is cyclophosphamide (NSC-26271): 20 mg/kg/injection, daily, days 1–11. Check control deaths, no-takes, etc.

Evaluation

Compute mean animal weight when appropriate, and at the completion of testing, compute T/C for all nontoxic test groups. When the parameter is tumor weight, a reproducible T/C $\leq$42% is considered necessary to demonstrate activity. When the parameter is survival time, a reproducible T/C $\geq$ 125% is considered necessary to demonstrate activity. For confirmed activity, a synthetic must have activity in two multidose assays (each performed at a different laboratory); a natural product must have activity in two different samples.

Reporting

On the final day of testing, prepare final control and test reports and send them for key-punching.

Walker Carcinoma 256

Tumor may be implanted sc in the axillary region as a 2- to 6-mm fragment, im in the thigh as a 0.2-ml inoculum of tumor homogenate containing 10^6 viable cells, or ip as a 0.1-ml suspension containing 10^6 viable cells. Drug treatment is usually ip. Procedures in this Protocol may vary by instruction from DR&D. Origin of tumor: arose spontaneously in 1928 in the region of the mammary gland of a pregnant albino rat (*5*).

Animals

Propagation: Outbred albino Sprague-Dawley rats.
Testing: F344 rats or outbred albino rats.
Weight range: 50–70 g (maximum of 10-g weight range within each experiment).
Sex: One sex used for all test and control animals in one experiment.

Experiment size

General testing: Six animals per test group.
Control groups: Number of animals varies according to number of test groups.

Time of tumor transfer

Time of transfer for propagation: Day 7 for im or ip implant; days 11–13 for sc implant.

Time of transfer for testing: Day 7 for im or ip implant; days 11–13 for sc implant.

Tumor transfer

Sc fragment implant is by trocar or 12-gauge needle into axillary region with puncture in inguinal area; im implant is with 0.2 ml of tumor homogenate (containing 10^6 viable cells) into the thigh; ip implant is with 0.1 ml of suspension (containing 10^6 viable cells) into the ip cavity.

Testing schedule

Prepare and administer drugs, weigh animals, and evaluate test on the days listed in the following table:

Test system	Prepare drug	Administer drug	Weigh animals	Evaluate
5WA16	2	3–6	3 and 7	7
5WA12	0	1–5	1 and 5	10–14
5WA31	0	1–9	1 and 5	30

Day 0: Implant tumor. Run bacterial cultures. Determine solubilities. Thaw solutions. Run positive control in every odd-numbered experiment. Record survivors daily.
Day 1: Check cultures. Discard contaminated groups. Weigh and randomize animals.
Day 2: Recheck cultures. Discontinue testing if contaminated.
Final day: Kill all survivors and evaluate experiment.

Quality control

Acceptable control weight or survival time for the following Walker 256 test systems:
5WA16: 3–12 g.
5WA12: 3–12 g.
5WA31 or 5WA21: 5–9 days.
Check control deaths, no-takes, etc.
Positive control compound is cyclophosphamide (NSC-26271): 2.5 mg/kg/injection.

Evaluation

Compute mean animal weight as appropriate, and at the completion of testing compute T/C for all nontoxic assay groups. When the parameter is tumor weight, a reproducible T/C less than or equal to 42% is considered necessary to demonstrate activity. When the parameter is survival time, a reproducible T/C greater than or equal to 125% is considered necessary to demonstrate activity. For confirmed activity, a synthetic must have activity in two multidose assays (each performed at a different laboratory); a natural product must have activity with two different samples.

Reporting

On the final day of testing, prepare final control and test reports and send them for key-punching.

B. PROTOCOL FOR IN VITRO KB CELL CULTURE SCREEN

KB cells are cultivated on Eagle's Basal Medium plus 10% serum. Stock cells are fed 24 hours before testing. Test material added on days 0 or 1. Results are expressed as the dose that inhibits growth to 50% of control growth by 3 days after drug addition. Origin of tumor line: derived from a human epidermoid carcinoma of the mouth (*6*).

Experiment size

General testing: three to five dose levels per material. Two tubes per dose level.

Control group: Number varies according to number of test groups (n), according to the formula: $2\sqrt{n}$. Determine base-line protein according to method of Oyama and Eagle (*7*).

Test schedule

Day 0: Dilute stock cells to 10–20 μg/ml (20,000–30,000 cells/ml) in complete media. Add cells to tubes and add test material simultaneously or on day 1. Total volume is approximately 3–4 ml. Run positive control on odd-numbered control groups.

Day 1: If 24-hour culture is used, refeed and add test material. Determine protein values of base-line tubes.

Day 3: Conduct protein analysis of test, control, and at least three protein standard and media blank tubes.

Day 4: If 24-hour cultures are used, conduct protein analysis as prescribed for day 3.

Dosage

Test synthetics and plant products by weight (W) at 100, 10, and 1 μg/ml.

Test crude fermentation products by dilution (D) at 1:10, 1:100, and 1:1,000.

Test dried or crystalline fermentation products by weight at appropriate concentrations. Any material which does not reach an end point at these levels is to be retested at lower concentrations. All additional tests are to be performed at five dose levels at 0.3-log intervals.

Quality control

Control tubes must show growth of at least six times that of base-line tubes. Positive control, 6-mercaptopurine (NSC-755), limits ED50 between 0.05 and 0.5 μg/ml.

Reporting

Mail test and control screening reports for data processing.

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(*1*) Law LW, Dunn TB, Boyle PJ, et al: Observations on the effect of a folic-acid antagonist on transplantable lymphoid leukemias in mice. J Natl Cancer Inst 10:179–192, 1949

(*2*) Dawe CJ, Potter M: Morphologic and biologic progression of a lymphoid neoplasm of the mouse in vivo and in vitro. Scientific Proceedings, 54th Annual Meeting American Association of Pathologists and Bacteriologists. Am J Pathol 33:3, 603, 1957

(*3*) Handbook on Genetically Standardized Jax Mice. Roscoe B. Jackson Memorial Laboratory. Bar Harbor, Maine, 1962. [*See also* Conference on the Biology of Normal and Typical Pigment Cell Growth of 1961. Ann NY Acad Sci 100 (Parts 1 and 2), 1963]

(*4*) Sugiura K, Stock CC: Studies in a tumor spectrum III the effect of phosphoramides on the growth of a variety of mouse and rat tumors. Cancer Res 15:38–51, 1955

(*5*) Durham LJ: A survey of transplantable and transmissible animal tumors. J Natl Cancer Inst 13:1299–1397, 1953

(*6*) Eagle H: Propagation in a fluid medium of a human epidermoid carcinoma, Strain KB. Proc Soc Exp Biol Med 89:362–364, 1955

(*7*) Oyama VI, Eagle H: Measurement of cell growth in tissue culture with a phenol reagent (Folin-Ciocalteau). Proc Soc Exp Biol Med 91:305–307, 1956

		Criteria of activity	
Assay	Drugs	First test[a]	Average (first and second tests)[a]
Sequential	Synthetics	ED50 $\leq 6\,\mu$g/ml	ED50 $\leq 4\,\mu$g/ml
	Plant extracts	ED50 $\leq 30\,\mu$g/ml	ED50 $\leq 20\,\mu$g/ml
	Fermentation products	ED50 $>$1:100 dilution	ED50 $>$1:100 dilution
Confirmation	Synthetics	ED50 $\leq 4\,\mu$g/ml	
	Plant and animal extracts	ED50 $\leq 20\,\mu$g/ml	
	Fermentation products	ED50 $>$1:500 if known classes of cytotoxic agents are excluded	

[a] ED = effective dose.

C. MODIFIED PROTOCOL FOR THE TESTING OF NEW SYNTHETICS IN THE L1210 LYMPHOID LEUKEMIA MURINE MODEL IN THE DR&D PROGRAM, DCT, NCI[1]

R. I. Geran, N. H. Greenberg, M. M. Macdonald, *and* B. J. Abbott[2]

The purpose of this now-modified Protocol (table 1) is to select synthetics with possible antitumor activity by using an initial three-mouse prescreen (stage I, step 1) to provide in vivo evaluation of as many compounds as possible, even those received in a small quantity, and by continuing the testing of any initially active drug with more mice per test and additional regimens.

The reduction in T/C value from 125 to 120% for rating activity will provide for capturing additional active compounds in the prescreen with the knowledge that the number of false positives will increase with the reduced percent T/C. These false positives will be eliminated at later screening stages. Any synthetic showing activity in the original three-mouse assay is to be retested in the originally active regimen and tested by as many of the other three regimens as possible with available supply. If the supply is not sufficient for all of this testing, retesting in the originally active regimen takes precedence and a refill is not to be requested unless activity is seen in two separate experiments.

Text-figure 1 is an outline of the general plan for stage I testing of a synthetic and table 2 is a list of the activity criteria for the various stages of testing.

The screening laboratory is to attempt to achieve maximum utilization of each compound, exercising judgment and modifying doses as experience with a compound accumulates. These comments pertaining to the current protocol are offered merely as guidelines.

A. Unless otherwise instructed, September 1972 protocols [3] still apply.
   Examples:
   1. When toxicity data are supplied, initial doses should be based on that information.
   2. For a multidose assay to be considered "inactive and complete" there must be at least two nontoxic inactive dose levels.

B. In stages I and II, a dose of 600 mg/kg/injection is not to be exceeded (tables 3, 4).

C. "Confirmed activity" is the required percent T/C in two separate experiments versus the same five-digit test system by the same drug route and drug schedule, either at the original laboratory or at another laboratory.

D. "Reproduced activity" is the required percent T/C in two separate experiments versus the same five-digit test system not by the same drug route and schedule, either at the original laboratory or at another laboratory.

E. If supply does not permit all testing, confirmation of previous activity takes precedence.

F. Rule-of-thumb factors to use in adjusting dose levels to new regimens are: one single=four intermittent=eight chronic (i.e., 1S=4I=8C). The following is an example of this relationship.

Regimen	Top dose, mg/kg/injection[a]
Single (S)	400
Intermittent (I)	100
Chronic (C)	50

[a] In no case is a 600-mg/kg/injection dose to be exceeded.

G. If a material is active, the animal weight change difference [AWCD(T-C)] is ignored, but a negative percent T/C with AWCD (T-C) greater than or equal to 4 g is considered toxic.

TABLE 1.—*Regimens and dose levels for stage I, step 1 testing*

Amount available, mg	Regimen	Doses, mg/kg/injection	Amount for step 1, mg
≥ 810	Every day, days 1-9	200, 100, 50	237
540-809	Every 4 days, days 1, 5, 9	400, 200, 100	158
360-539	Every 4 days, days 1, 5	400, 200, 100	105
180-359	Day 1 only	400, 200, 100	53
90-179	Day 1 only	400	30
45-89	Day 1 only	200	15
15-44	Day 1 only	200	15

TABLE 2.—*List of activity criteria for synthetics*[a]

Criterion of activity	Disease	Percent ILS	Confirmed percent ILS
In stage I testing	L1210 leukemia	20	
In stages II or III testing (one)	L1210 leukemia	25 (average)	
	P388 leukemia	25	
	B16 melanoma	40	
	Lewis lung carcinoma	40	
Required for candidate for DN-II[b] (one)	L1210 leukemia	5	50
	P388 leukemia		75
	B16 melanoma		50
	Lewis lung carcinoma		50

Note that stage II testing is started by the screening laboratory when a material meets the requirement for a material classification code "OS"; *see* table 3.

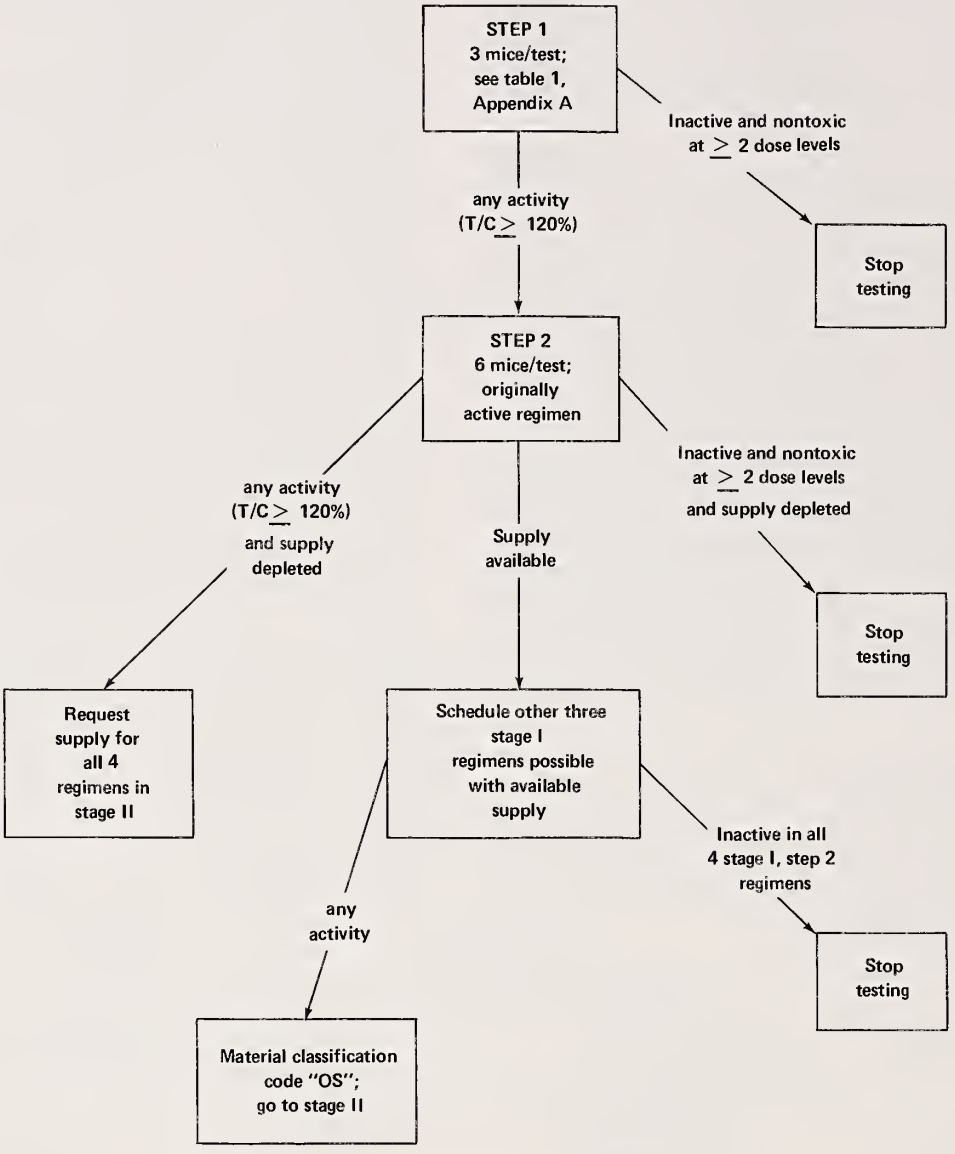
[a] ILS = increase in life-span.

[b] DN = Decision Network.

[1] Drug Research and Development (DR&D) Program, Division of Cancer Treatment (DCT), National Cancer Institute (NCI).

[2] Drug Evaluation Branch, DR&D, DCT, NCI, Bethesda, Md.

[3] Cancer Chemother Rep (Part 3) 3:1-102, 1972.



TEXT-FIGURE 1.—General plan for testing a synthetic in stage I.

H. If any synthetic has reproduced activity (T/C greater than or equal to 120%), all stage II regimens are to be tested that were not tested in stage I. If a supply is not available for all regimens, the screener is to request a refill through the screening services contractor.
I. In vivo screening is now to be scheduled for all compounds of which at least 15 mg is received.
J. No material is to be transshipped by the original laboratory for in vivo confirmation except on specific request.
K. Synthetics received in quantities of 179 mg or less that are negative in stage I, step 1 should be routinely transshipped by the in vivo screening laboratory for KB testing if the supply permits. No other synthetic is to be transshipped for KB testing unless transshipment is specifically requested.

Any material active in the three-mouse assay (stage I, step 1) but inactive in the first repeat six-mouse assay (same regimen) is considered complete unless quantity permits testing by other schedules. No request for a refill is to be made for additional schedules.

TABLE 3.—*Stage I screen for synthetics*

For all of the steps in stage I screen for synthetics, use
Tumor: L1210 implanted ip (3LE21)
Drug route: ip
Parameter: mean survival time
Criterion of activity: T/C $\geq$ 120%.

Stage I, step 1 (initial testing)
No. of mice/test: 3
No. of regimens: 1
No. of doses: 3 (when quantity permits)
Dose levels and regimen: (*see* table 1)

Follow-up: if supply is sufficient, go to stage I, step 2a; if supply is insufficient for stage I, step 2a, go to stage I, step 2b

Stage I, step 2a (repeat of stage I, step 1 with adequate supply)
No. of mice/test: 6
No. of regimens: from 2 to 4
No. of doses: 4—$2x$, x, $0.5x$, and $0.25x$ where x = optimal dose from stage I, step 1; an exception occurs when original material received is $\leq$ 179 mg (*see* comment F regarding regimen factor)
Regimens: the same as stage I, step 1 plus any or all of the others listed in table 1 permissible with available supply

Stage I, step 2b (repeat of stage I, step 1 with limited supply)
No. of mice/test: 6
No. of regimens: 1
No. of doses: 4—$2x$, x, $0.5x$, and $0.25x$ where x = optimal dose in stage I, step 1
Regimen: same as stage I, step 1
Criterion for passing stage I (material classification code "OS"): a T/C $\geq$ 120% in a three-mouse test (any regimen) plus a T/C $\geq$ 120% in a six-mouse test (any regimen)

If a material is active in the three-mouse assay and the quantity is not sufficient (QNS) to repeat with 6 mice the regimen in which activity was found, a refill should be requested.

If a material shows reproduced activity in stage I, step 2 and quantity is insufficient for all stage II testing, a refill should be requested.

Any material in insufficient quantity for stage III testing (table 5) will be reported QNS by the Drug Evaluation Branch staff, who will have responsibility for scheduling this testing.

TABLE 4.—*Stage II screen for synthetics*

Test system: L1210 implanted ip (3LE21)
No. of mice/test: 6
Drug route: ip
No. of regimens: 4
No. of doses: 5—⅔ intervals surrounding x where x = most effective dose from stage I (*see* comment F regarding regimen factor)
Regimens: single, day 1; every 4 days, days 1 and 5; every 4 days, days 1, 5, and 9; and every day, days 1-9. All regimens not tested in stage I *and* all regimens active at least once in stage I, must be tested in stage II.
Criteria for passing stage II: *a*) any one T/C $\geq$ 200%, or *b*) an average T/C $\geq$ 125% from the optimal responses (any regimen) in each of two six-mouse experiments with each T/C value used to calculate the average $\geq$120%.

TABLE 5.—*Stage III screen for synthetics and natural product isolates*

Input	
Analogue	Unique
1. Determine interest in type 2. Testing directed specifically to type; based on biologic, biochemical, pharmacologic, physical-chemical properties of parent	Type I schedule dependency (including oral and sc treatment, and toxicity in normal mice) Early 3PS31 (ip, P388 leukemia) Early 3B131 (ip, B16 melanoma) Early 3B132 (sc, B16 melanoma) Advanced 3LE32 (sc, L1210 leukemia) Advanced 3PS32 (sc, P388 leukemia) Early 3LL32 (sc, Lewis lung carcinoma) and, if active, advanced 3LL32 Early 3LE37 (ic, L1210 leukemia), 3PS37 (ic, P388 leukemia), 3B137 (ic, B16 melanoma), or 3EM37 (ic, ependymoblastoma) Other secondary screens

D. MEMORANDUM TO SUPPLIERS OF COMPOUNDS, FEBRUARY 13, 1976

S. A. Schepartz[1]

In 1976, the Division of Cancer Treatment (DCT) intends to place greater emphasis on the testing of new synthetic compounds and natural product isolates against a spectrum of animal models of specific human tumors. Although the selection of all systems has not yet been accomplished, it is expected that this panel of animal screens (*see* text-fig. 2) will include mouse colon, breast, and lung tumors. Human tumor xenografts in athymic mice will be included as the supply of these immunologically incompetent animals and testing capacity permit. Other animal tumors of specific interest, e.g., transplantable brain tumors, will be utilized in specific instances. The level of effort required to test a material in most of the systems in the spectrum is greater than the present effort for primary screening against transplantable mouse leukemias. This and the number of test systems involved means that the input to this panel of antitumor screens will be limited in number. This raises the obvious question of the basis for selecting materials for such broad spectrum screening.

After considering a number of alternatives, we have determined that the most reasonable approach would be to utilize, as an initial in vivo screen or prescreen, one which has demonstrated sensitivity to most classes of clinically effective anticancer drugs, but which, nevertheless, is sufficiently discriminating to avoid "overloading" the spectrum. The current screen for synthetic agents, mouse leukemia L1210, though known to be highly predictive for clinical utility against human leukemias, lymphomas, and solid tumors, was considered to be too restrictive for a prescreen designed to minimize the chance of missing a compound likely to show activity in one of the tumors in the spectrum. A comprehensive review of existing screening data indicated that mouse leukemia P388 would present a more appropriate prescreen because *a*) its response to drugs of various classes is qualitatively similar to that of L1210, and *b*) it is quantitatively more sensitive than L1210.

The objective of the prescreen, which must be limited to one in vivo system because of cost considerations, is to select a population of materials that will yield a higher percentage of "actives" in one or more of the spectrum tumors than would de novo screening in the entire spectrum. In addition, it is expected that the prescreen will include an in vitro assay which will permit the selection, for subsequent in vivo spectrum screening, of highly cytotoxic materials and materials which may induce cellular differentiation, a potentially useful drug action that cannot be discerned with the present in vivo models or the KB cell culture screen. At this time, we are investigating the Friend virus-induced erythroleukemia in vitro for its utility for this purpose.

Crude natural products will continue to be tested against KB cells in culture and against P388 in mice. The KB cell culture screen has been an efficient tool for bioassay of natural product fractions that show activity both in vivo and in vitro, and there is no reason to alter the current practice for these materials. Active isolates from the natural produce screen will be tested against the tumor spectrum.

Despite the necessity of limiting our initial in vivo screen to one assay, we recognize that no animal model is known to predict perfectly for clinical utility. Therefore, we plan to augment the input to the tumor spectrum with materials with antitumor activity reported in the world's literature and from other screening programs, and with compounds reported to exhibit pertinent biochemical and other biologic activities. Such a compound will be tested in the DCT prescreens to provide data for future analyses but will be tested in the tumor spectrum regardless of prescreening results. In a sense then, agents which have shown interesting activity outside of our program will essentially "by-pass" the prescreen as a barrier to broad spectrum screening.

Because of their demonstrated past utility, leukemia L1210 and B16 melanoma will be retained as part of the screening spectrum. Lewis lung carcinoma is still being used but alternative mouse lung tumor systems are being investigated. Congeners of known active drugs will continue to be tested in the specialized assays designed for each structural class.

As materials emerging as active from any of the spectrum models (or even very active in the P388 screen) are subjected to adequate clinical trial, this approach to screening will provide, prospectively, critical information relative to the value of specific animal tumors or combinations of animal tumors.

As the DCT panel of antitumor screens is phased into use, it is expected that some preselection or priority rating, even for the prescreen, will have to be made. Logical bases for preselection are under discussion, but it is reasonably certain that, in the absence of demonstrated biologic or biochemical data of some kind, structural considerations will weigh heavily.

In attempting to establish priorities for screening, information from the supplier will be invaluable. Therefore, if you have data to suggest that a specific compound or group of compounds might possess a greater-than-average probability of being active in the screens, such information should be brought to our attention. Also, information on toxicity in animals and solubility in physiologically compatible vehicles will eliminate a great deal of retesting and waste of compound.

In the near future, you will begin to receive results in P388 and gradually in other systems. If you have any questions concerning the interpretation of data or the overall approach, please contact us. As future developments occur in the screening program, we will contine to keep you informed.

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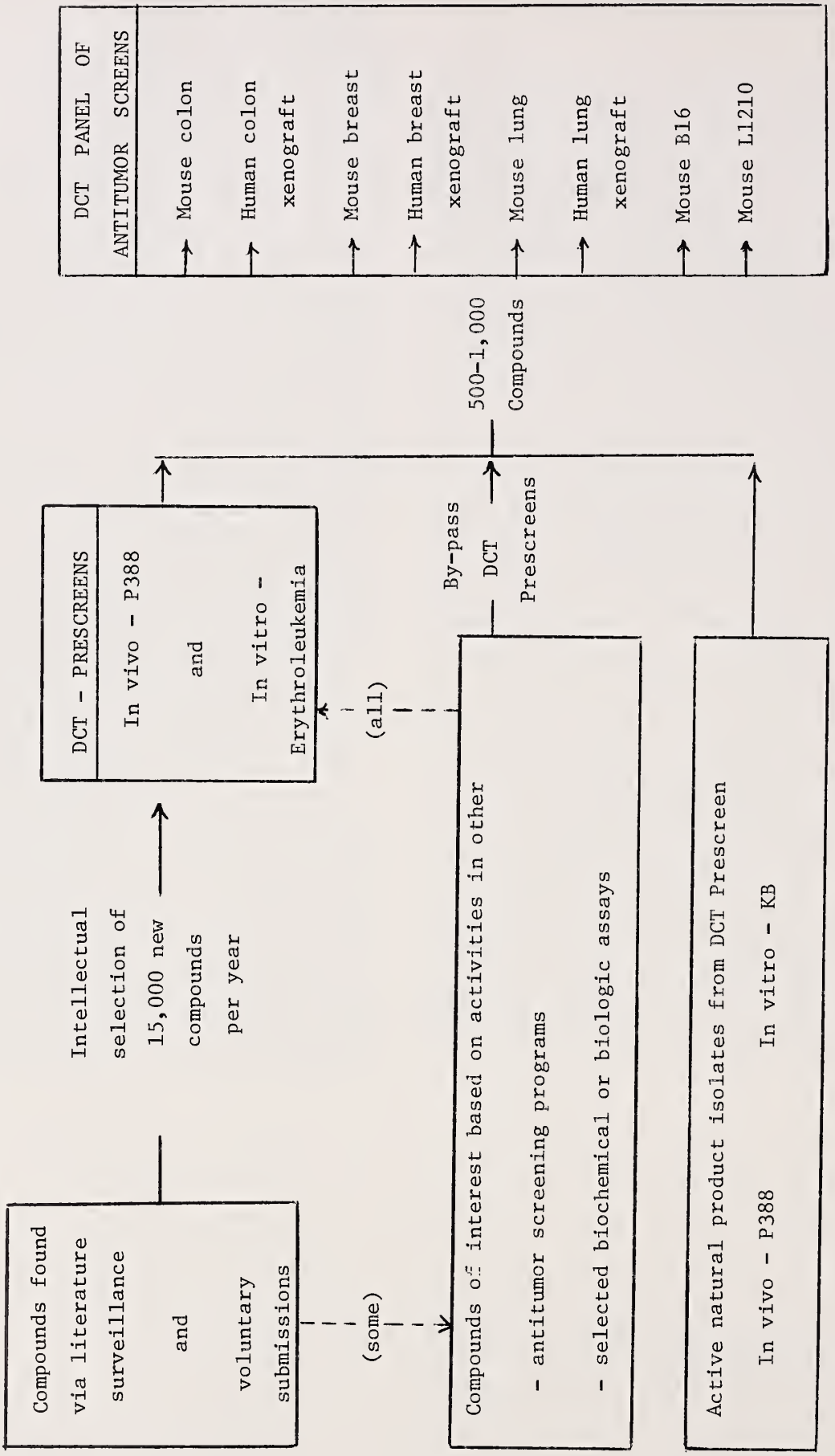
[1] Associate Director for Drug Research and Development, Division of Cancer Treatment, National Cancer Institute.



TEXT-FIGURE 2.—Proposed flow of drugs through DCT screens.

APPENDIX IV

Preclinical Toxicology Protocols of the Laboratory of Toxicology[1]

David J. Prieur, David M. Young, Ruth D. Davis, David A. Cooney, *and* Anthony M. Guarino[2]

The qualitative or quantitative extrapolation of preclinical data to man and the accuracy of predicting toxicologic effects in the clinic depend in part on the degree of correlation between the responses of the laboratory species and the responses of man (*1, 2*). Although preclinical toxicologic studies conducted in several species have served well to reveal the specific test parameters that act as indicators of toxicity in man, it is to be expected that unique or unusual toxic effects may remain undetected because of limitations of observations or inherent species differences (*3*).

Once a toxic effect in an organ system is observed during an animal study, it should be viewed with a realistic understanding of the following limitations of animal toxicologic data:

1) The toxic effect may develop in man in an organ system predicted to be susceptible from the animal data, but may be expressed in a different specific clinical, morphologic, or chemical parameter.
2) The adverse reaction may appear in man at a greater or lesser dose level.
3) The toxic effect may follow a different order of appearance in relation to the total spectrum of qualitative toxic effects inherent in any compound.
4) The toxic effect may not develop in man (*3*).

The effective use of animal toxicologic data, coupled with careful monitoring, considered judgment, and expectation, serve to forewarn the physician of the development of critical organ system toxic effects during an initial clinical drug trial.

The minimal protocol study for a candidate drug includes the following:

1) Single-dose study (dogs)
2) Five consecutive daily treatments (dogs)
3) Five consecutive daily treatments (monkeys)
4) Five consecutive daily treatments, 9-day rest, repeated for three treatment periods (dogs)
5) Schedule-dependency studies (dogs) using at least one of the following:
   a) Weekly 48-hour iv infusions for 6 weeks, or
   b) Treatment every 6 hours for 42 hours per week for 6 weeks, or
   c) Weekly treatment for 6 weeks, or
   d) Ten consecutive daily treatments.

PROTOCOL REQUIREMENTS

A. General Requirements

1) The test material to be evaluated will be authorized and furnished by the Laboratory of Toxicology, DCT, NCI. After the studies are completed, surplus materials will be returned as directed.
2) The contractor shall not disclose, directly or indirectly, the identity of the substances under test or the results of the tests performed to any person other than authorized personnel of the DCT, NCI, except after written authorization from the project officer.
3) The Laboratory of Toxicology will provide the contractor with the available toxicologic data that concerns the drug to be evaluated. If unexpected adverse effects are observed at any stage during the toxicologic testing of a compound, the principal investigator must telephone the project officer. In addition, an explanatory letter should be sent to the Laboratory of Toxicology.
4) If modifications in the protocols or supplementary studies are needed, they must be approved by the project officer.
5) When an animal dies after treatment with a dose of drug, which was nonlethal at a higher dose (e.g., 4 mg/kg was lethal, 2 mg/kg was toxic but not lethal, and subsequently 1 of the 2 animals treated with 1 mg/kg dies), a pair of animals should be retested at this dose level and the cause of death should be determined. If neither of the substituted pair of animals dies, the dose should be considered nonlethal; but if one or both of the substituted animals dies, the dose should be considered lethal.

Potentially useful cancer chemotherapeutic agents are selected through the drug evaluation procedures of the Drug Research and Development (DR&D) area of the Division of Cancer Treatment (DCT), National Cancer Institute (NCI). Before a new chemotherapeutic agent is considered for initial clinical trials, however, precise knowledge of its toxicologic potential must be developed by systematic studies in laboratory animals. This outline of procedures is the guide followed by the contractors of the Laboratory of Toxicology, DCT, NCI, in the toxicologic characterization of candidate antineoplastic agents.

No single scheme of operation can set a priori limits to the objectives of preclinical toxicologic studies, and it would not be appropriate to set rigid specifications or criteria which apply uniformly to all experimental drugs. Nevertheless, an attempt should be made to outline basic procedures that furnish adequate and useful toxicologic data aimed at defining the probable risks involved during clinical trials. It is anticipated that the test procedures will be applied with judgment and that necessary modifications will be introduced as indicated by preliminary findings. The principal investigator of each contract, in consultation with the NCI project officer, is expected to use techniques that are best suited to delineate specific toxicologic aspects, even though individual procedures may not be described in the protocol.

6) Standard laboratory and analytical procedures utilizing microtechniques are to be employed whenever possible.

[1] Laboratory of Toxicology, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014.

[2] The authors express their sincere appreciation to Mrs. Linda Klipp, Mrs. Audrey Loveridge, Mrs. Emily Olausen, and Mrs. Terri Wise for secretarial assistance.

Each contracting laboratory will maintain a continuing record of control values for each test species.

7) All doses are to be expressed as milligrams per kilogram of body weight and milligrams per square meter of body surface. For the Laboratory of Toxicology the (dose in mg/m^2) = (dose in mg/kg) × (S), where S is a species factor; S = 3 in mice, 6 in rats, 7 in guinea pigs, 11 in rabbits, 12 in monkeys, 20 in dogs, and 37 in man (*1*).

B. Animal Facilities

1) The animal facilities must comply with Public Law 89–544, "The Laboratory Animal Welfare Act of August 24, 1966," as amended by Public Law 91–579 of December 24, 1970.
2) Because of the effects of chlorinated hydrocarbons on drug metabolism, no insecticides of this type should be used in the animal facilities (*4*).

C. Dogs

1) Beagles (8–12 months of age) are to be used in the toxicity studies.
2) Beagles will be supplied without charge to the contractor by the Mammalian Genetics and Animal Production Section, DR&D, DCT, NCI.
3) The dogs will be vaccinated against canine distemper, infectious canine hepatitis, and leptospirosis before shipment to the contractor.
4) The contractor will hold dogs in quarantine a minimum of 2 weeks before initiation of toxicologic studies.
5) If the dogs require parasite control measures, these should be performed at least 1 month prior to the time they are placed on study and should not include chlorinated hydrocarbons.
6) During the time the dogs are on experiment, they should not be treated with any other drug, either for manifestations of disease apparently unrelated to the anticancer agent or for those manifestations of disease which are obviously related to toxicity of the drug, unless specifically requested by the project officer.
7) A male and a female are to be used at each dose level.
8) Approximately 36 dogs will be required for the entire set of preclinical toxicologic studies.

D. Monkeys

1) Rhesus (*Macaca mulatta*) monkeys (2–5 kg) are to be used in the toxicity studies.
2) It is anticipated that these monkeys will be supplied, without charge to the contractor, by the National Institutes of Health (NIH).
3) Monkeys will be tuberculosis (TB) tested just prior to shipment.
4) Monkeys are to be held in quarantine for 2 weeks before the initial TB testing and are to be tested every 2 weeks thereafter for a minimum of three tests before beginning toxicologic studies.
5) Monkeys reacting positively to TB testing should be killed and the supplier should be notified.
6) The monkeys are not to be placed on test until they have been held in quarantine for a minimum of 6 weeks.
7) The above comments concerning parasite control measures and the use of other drugs in dogs also apply to monkeys.
8) A male and a female are to be used at each dose level.
9) Approximately 10 to 12 monkeys will be required for the entire set of preclinical toxicologic studies.

DEFINITIONS

The following definitions of the levels of toxicity are used by the Laboratory of Toxicology:

A. Highest Nontoxic Dose (HNTD)

The highest dose at which no hematologic, chemical, clinical, or morphologic drug-induced alterations occur; doubling this dose produces the aforementioned alterations.

B. Toxic Dose Low (TDL)

The lowest dose which produces drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose produces no lethality.

C. Toxic Dose High (TDH)

The dose which produces drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose produces lethality.

D. Lethal Dose (LD)

The lowest dose which produces drug-induced death in any animal during the treatment or observation period.

DOG AND MONKEY STUDIES

An outline of the preclinical toxicity studies involving dogs and monkeys is provided in table 1. The specific information pertaining to each study is included in the text under the appropriate heading. The initial dose, route of administration, and dosage formulation for these studies will be specified by the project officer. The rate of iv injection will depend on the type of reaction to the compound; therefore, slow infusion studies may be requested in certain instances. If the drug is to be administered orally, the animals should be fasted for 12 hours prior to dosing. Oral doses are to be administered in gelatin capsules whenever feasible. If necessary, a uniformly fine suspension of drug in an appropriate suspending medium may be administered by gavage. In studies in which new or unusual vehicles or excessive dose volumes are required, the project officer will direct the contractor to include vehicle-treated control animals. A complete necropsy must be performed on each animal according to the procedures given in the pathology section.

A. Study I: Single-Dose Study in Dogs

1) Purpose
   a) To determine the toxic effects of graded dose levels of a drug after single treatments in dogs.
   b) To establish the HNTD, TDL, TDH, and LD in dogs for single-dose treatments.
   c) To identify specific test parameters that indicate abnormal conditions and to establish tests which might predict toxic drug effects in man.
2) Procedures
   a) Perform routine ophthalmoscopic examination (*5*), urinalysis, and hematologic and clinical tests (forms

1–3) on each dog once 8–10 days before and once within 3 days before the initiation of treatment. Use only those dogs whose values for each parameter fall within accepted norms.

b) Administer the drug to a male and female dog with the dose and route specified by the project officer. Observe and record signs of toxicity (form 3) and perform indicated clinical tests listed below (sections "*h*" and/or "*i*").

c) Place additional animals (male and female) on test at half or twice the original dose level depending on response of the first animals. Doses must range from highest nontoxic to lethal (HNTD, TDL, TDH, LD) and should be escalated and/or reduced geometrically (1, 2, 4, 8, etc.).

d) Observe and record food and water intake daily for the first week.

e) Record body weight twice a week.

f) Record clinical signs (vomiting, diarrhea, polyuria, lacrimation, lethargy, etc.) throughout the course of the study.

g) Record body temperature immediately before treatment and 1 and 3 hours after treatment. Thereafter record temperature weekly or as the clinical signs warrant.

h) Perform tests for blood urea nitrogen, alkaline phosphatase, serum glutamic oxaloacetic transaminase, packed cell volume, total and differential white blood cells, and platelets every other day during the first week (Abbreviated Test Series).

i) Perform a complete set of hematologic and chemical parameters (form 2) on day 8 and weekly thereafter. Perform additional tests as indicated by the observed toxicity.

j) Obtain a scrupulously clean urine specimen with a sterile catheter from each dog once per week after treatment and perform a urinalysis (table 1) on each urine sample.

k) Kill moribund animals by either barbiturate overdose or electrocution.

l) Retain 1 dog at each dose level (if possible) for a 45-day observation period. Kill the other dogs on day 8. As a general rule, kill the dog with the more severe signs of toxicity on day 8 and allow the less severely affected dog to live through the observation period. If the dog killed on day 8 is moribund, consider the dose it received a lethal dose.

m) Kill the remaining dogs at the end of the 45-day observation (recovery) period, provided that there is some evidence the observed toxicity has been reversed. Otherwise, extend the observation period until adequate hematologic or chemical evidence of reversibility or irreversibility is obtained.

n) Submit a complete report of this study, including raw data and pathologic findings with the final report on forms similar to forms 1–5.

B. Study II: Daily Treatment of Dogs for 5 Consecutive Days

1) Purpose

a) To determine the toxic effects in dogs after repeated administration of drug.

b) To establish the HNTD, TDL, TDH, and LD in dogs after 5 consecutive daily treatments.

c) To identify specific test parameters and establish tests which may predict adequately the toxic effects in man.

2) Procedures

a) Administer the initial dose to 1 male and 1 female dog using the TDL established in study I.

b) Administer 5 consecutive daily doses to dogs on this study adjusting each daily dose to correspond to any change in body weight.

c) Except under the following stipulations perform the studies in the same manner as in study I:

i) Kill the more severely affected dog on day 6 (24 hr after the last treatment) and retain the other dog for a 45-day period of observation after the last dose at each dose level.

ii) Submit a pair of untreated control dogs (1 male and 1 female) to the same handling, series of test procedures, and day of death as the treated dogs.

iii) Perform urinalyses on the dogs in study II only if the principal investigator in consultation with the project officer determines that there is adequate evidence of renal toxicity in study I.

C. Study III: Daily Treatment of Monkeys for 5 Consecutive Days

1) Purpose

a) To determine the toxic effects in monkeys of repeated administration of drug.

b) To determine the species specificity of any of the toxic changes in dogs by observation of the effects of the drug in a second species.

c) To establish the HNTD, TDL, TDH, and LD in monkeys for 5 consecutive daily treatments.

2) Procedures

a) Administer the initial dose of the drug daily for 5 days to 1 male and 1 female monkey, with the dose in milligrams per square meter established as the TDH in study II.

b) Perform the same procedures on the monkeys as those used in study II including the use of untreated controls.

D. Study IV: Daily Treatment of Dogs for 5 Days, 9-Day Rest, Repeated for Three Treatment Periods

1) Purpose

a) To determine if the toxic effects of treatment are reversible during a 9-day period of nontreatment.

b) To determine if the toxic effects of 5-day treatments are cumulative when three such treatment periods are each separated by 9 days.

2) Procedures

a) Treat a total of 6 dogs, 2 (1 male and 1 female) at each of the defined nonlethal doses (HNTD, TDL, and TDH) established in study II.

b) If no TDL has been established in study II (if there is only one dose level between the HNTD and LD), use just two pairs of dogs, one pair at the HNTD and one at the TDH dose level.

c) Treat each dog for 5 consecutive days followed by 9 days of rest. Repeat the treatment for a total of three treatment periods.

d) Kill one of each pair of dogs 1 and 45 days after the last treatment (days 34 and 78).

e) The rest of the study will duplicate study II except for the following changes in the days of examination:

i) The Abbreviated Test Series is to be performed on

the second and fourth day during each treatment period and the fourth and sixth day of each rest period.

ii) Perform a complete test series just before (same day) the first treatment of each series of treatments and weekly after the completion of the treatment regimen.

E. Study V: Schedule-Dependency Studies in Dogs

1) Purpose
   a) To determine the possible existence of significant differences in toxicity associated with schedule dependency as indicated by antitumor screening data.
   b) To obtain additional data for the correlation of various dose levels on different schedules in order to predict more accurately the quantitative toxicity in man.
2) Procedures
   a) Although four studies are outlined, only one or two of the four schedules will be routinely requested by the project officer. However, occasionally, the project officer may request a schedule-dependency study not listed below.
   b) *Study V-A*
      i) Perform 48-hour iv infusions of the drug once a week for 6 weeks. Treat 2 dogs (1 male and 1 female) with the TDL and 2 dogs with the TDH established in study I.
      ii) Perform an Abbreviated Test Series on the third and fifth days after the initiation of each infusion (e.g., when the infusion is started on Monday perform an Abbreviated Test Series on Wednesday and Friday).
      iii) Perform a complete series of tests immediately before (same day) the start of the next infusion and weekly during the observation period.
      iv) Kill 1 of the dogs at each dose level on day 39 and the other on day 83.
      v) Other test procedures should duplicate those in study II except that no untreated controls will be used.
   c) *Study V-B*
      i) Treat the dogs with one-eighth the TDL and TDH from study I every sixth hour for 42 hours (8 treatments/42 hr) once a week for 6 weeks by the same route of administration. (Divide the TDL and TDH from study I into eight equal parts and administer one part every 6 hr.)
      ii) Perform an Abbreviated Test Series on the third and fifth days after the initiation of each series of treatments (e.g., when a series of treatments is started on a Monday, perform an Abbreviated Test Series on Wednesday and Friday).
      iii) Perform a complete series of tests immediately before (same day) the start of the next series of treatments and weekly during the observation period.
      iv) Kill 1 of the dogs at each dose level on day 39 and the other on day 83.
      v) Other test procedures should duplicate those in study II.
   d) *Study V-C*
      i) Treat 2 dogs (1 male and 1 female) with the TDL and 2 dogs with the TDH established in study I. Use a single dose once a week for 6 weeks.
      ii) Kill 1 dog at each dose level on day 37 and the other dog on day 81.
      iii) The test procedures should duplicate those of study II except that no untreated controls are to be used.
   e) *Study V-D*
      i) Treat 4 pairs of dogs for 10 consecutive days with half the defined daily doses (HNTD, TDL, TDH, and LD) of study II (e.g., if 80, 40, 20, and 10 mg/kg/day are the respective LD, TDH, TDL, and HNTD in study II, administer 40, 20, 10, and 5 mg/kg/day by the same route of administration to 4 pairs of dogs for 10 consecutive days).
      ii) Adjust each daily dose to correspond to any change in body weight.
      iii) Kill the more severely affected dog in each pair on day 11 and the remaining dog on day 55.
      iv) The test procedures should duplicate those of study II except that no untreated controls are to be used.

IDENTITY AND PURITY TESTS

A. Purpose

1) To check the identity of the test compound.
2) To determine the purity of the test compound.

B. Procedures

1) The required tests will be indicated by the project officer. They often include tests such as an infrared, ultraviolet, or nuclear magnetic resonance, spectral analysis, melting point determination, or elemental analysis.
2) The project officer should be notified of the results by telephone within a week of the receipt of the drug. The results should also be included in a confirmation letter to the project officer, and the detailed data should be included in the final report.

MOUSE LD50 STUDY

A. Purpose

1) To determine the mean lethal dose (LD50) in mice.
2) To develop data that will permit an estimation of drug toxicity.
3) To provide toxicity data from a rodent species for comparison with data from dogs and monkeys.

B. Procedures

1) Route(s) of drug administration will be specified by the project officer.
2) Select groups of CDF_1 mice weighing 21–25 g.
3) Mice will be supplied, without charge to the contractor, by the Mammalian Genetics and Animal Production Section, DR&D, DCT, NCI.
4) In the absence of any toxicity data, a pilot experiment involving 2 mice per group should be done with the standard log interval spacing (e.g., studies might start at 0.1 mg/kg and include other doses of 1, 10, 100, etc., until a definite point of toxicity is established).
5) Perform dose mortality experiments by treating 26 mice (13 males and 13 females) per dose level to determine the

LD50 and the slope of the dose–response curve. Ten males and 10 females per dose level are to be used in the calculation of the LD50. Three males and 3 females constitute separate satellite groups for necropsy if needed (*see* 7 below).

Record data on each sex separately. At least four dose levels must cover the range from 0–10 to 90–100% lethality (i.e., at least one dose level must have from 0–10% lethality, at least two dose levels must have from 11–89% lethality, and at least one dose level must have from 90–100% lethality). In general, the log interval between dose levels will be 0.1–0.3 unless otherwise indicated by the results of the preliminary tests.

6) Record all drug effects, recovery rates, days of deaths, body weight changes, and other pertinent toxicologic information.
7) Observe the mice daily for 14 days after treatment. Perform gross and histopathologic examinations (liver, kidney, lung, duodenum, heart, spleen, pancreas, and brain) on moribund mice (two per dose level) and on 2 mice per dose level on day 14. Necropsies must be performed immediately after death. If an animal dies and is not observed to die, an animal from the satellite group should be killed at that time and necropsied.
8) Calculate the 24-hour and 14-day LD90, LD50, LD10, slope of the log dose–probit response curve, and the 95% confidence limits according to the procedures described by Litchfield and Wilcoxon (*6*).
9) Submit detailed data on a form similar to form 6.

ANCILLARY STUDIES

One or more of the following studies will be requested occasionally by the project officer. Such requests will be based upon previous toxicologic data, structure or type of drug, route of administration, and other criteria. The following list is not inclusive and other types of studies will be requested when warranted.

A. Compatibility Testing of Dosage Formulation

1) Purpose
   a) To assess the compatibility of the formulated drug with serum and plasma.
   b) To assess the hemolytic potential of the formulated drug.
2) Procedures
   a) Mix the drug (formulated in the highest concentration that is anticipated to be used in the dog and monkey studies) with an equal volume of fresh canine serum. If a greater concentration of the drug is later utilized in the dog or monkey studies, repeat the compatibility tests using the higher concentration of formulated drug. If the combination is incompatible (e.g., precipitation or coagulation occurs), repeat the procedure with an equal volume of vehicle and serum without the addition of drug. Then repeat the procedures in which incompatibility occurred with half the amount of drug and/or vehicle (1 vol of formulated drug to 2 vol of serum and/or 1 vol of vehicle to 2 vol of serum). If this combination is incompatible, decrease by half the proportion of drug and vehicle until there is no incompatibility. Record the results on a form similar to form 7.
   b) Perform the same procedures with fresh canine plasma.
   c) Using fresh heparinized canine blood, set up four tubes containing: 1) a volume of formulated drug with vehicle and an equal volume of blood, 2) a volume of vehicle and an equal volume of blood, 3) a volume of blood and an equal volume of plasma, and 4) as a positive control, a volume of 1% saponin (1 g/100 ml 0.9% saline) and an equal volume of blood. Incubate these four tubes for 45 minutes at 37° C. Centrifuge the tubes at $1{,}000 \times g$ for 5 minutes. Quantitate the amount of hemoglobin in the supernatant plasma by the cyanmethemoglobin method (*7*). If the hemolysis of the drug–blood and/or the vehicle–blood combination is greater than that of the blood–plasma control, decrease the ratio by half until the amount of hemolysis is the same. Record the results on a form similar to form 7.

B. Local Tissue-Reaction Study

1) Purpose
   a) To provide a technique to test primarily those drugs intended for sc or im administration.
   b) To evaluate local tissue reactions after sc and im injection of the drug into guinea pigs and rabbits, respectively.
2) Procedures
   a) Guinea pig study
      i) In 5 ml of vehicle, dilute the weight of the drug per kilogram that was established as the TDH in study I (e.g., if 25 mg/kg was the TDH in study I, dilute 25 mg of drug in 5 ml of vehicle).
      ii) Into three separate subcutaneous abdominal areas of 2 white, mature, short-haired guinea pigs, inject 0.5 ml of the diluted drug (dd) and 0.5 ml of two serial 1:1 dilutions (i.e., dd/2 and dd/4). Do not shave or clip the hair of the guinea pigs. As a control, inject 0.5 ml of the vehicle into a fourth subcutaneous abdominal area of each guinea pig.
      iii) Kill guinea pigs on the fifth day after treatment.
      iv) Examine all the lesions grossly and record the size.
      v) Perform histopathologic examination of each of the lesions.
      vi) If the concentrations are lethal to the guinea pigs, inject each dose into a single guinea pig.
      vii) Report the results on a form similar to form 8.
   b) Rabbit study
      i) If this study is performed the same day as the guinea pig study, use the same diluted drug; otherwise, prepare fresh drug for this study.
      ii) Record the body temperature of the rabbits prior to treatment.
      iii) Inject 0.5 ml of the diluted drug and 0.5 ml of two serial 1:1 dilutions im (i.e., dd/2 and dd/4) into three separate areas of the right sacrospinalis muscle of a rabbit. As a control, inject 0.5 ml of the vehicle into the left muscle.
      iv) Inject 0.5 ml, 0.25 ml, and 0.125 ml of the original diluted drug im into the right sacrospinalis muscle of another rabbit.
      v) Record body temperature 1, 2, and 3 hours after treatment.
      vi) Kill the rabbits on the fifth day after treatment.
      vii) Remove the sacrospinalis muscles intact and fix in toto in 10% buffered neutral formalin.
      viii) Examine each lesion grossly after fixation and record the dimensions.

ix) Perform a histopathologic examination of specimens from each of the lesions.
x) If these concentrations are lethal to the rabbits, inject the doses into separate rabbits.
xi) Report the results on a form similar to form 8.

C. Antigenicity Tests

1) Purpose
a) To provide a technique to test those drugs that have chemical structures or other properties indicating they may be immunogenic.
b) To evaluate the capacity of a drug to sensitize guinea pigs and to sensitize and produce antibodies in rabbits.
2) Procedures
a) Capacity to sensitize guinea pigs.
i) Animals: 10 guinea pigs (5 of each sex), 200–300 g in weight; immunize 6 of the guinea pigs and hold 4 as controls.
ii) Dose: Use one-tenth the LD50 as determined in mice (as expressed in milligrams per kilogram).
iii) Immunization: Inject the dose by the ip route on Monday, Wednesday, and Friday of 1 week and on the following Monday.
iv) Sensitivity testing: Fourteen days after administration of the first dose of the drug, inject an equal dose of the drug either iv or intracardially into the 6 test and 4 control guinea pigs.
v) Interpretation: Signs of respiratory distress or death within 1 hour after the injection of the shocking dose of the antigen and the absence of such a response in the nonimmunized control guinea pigs are to be regarded as evidence of antigenicity on the part of the drug.
vi) Remarks: Because the guinea pig is uniquely sensitive to certain drugs, and in the absence of toxicity data in this species, it may be necessary to perform a range-finding study to determine the suitable dose of the drug for use in this test.
b) Capacity to sensitize rabbits.
i) Animals: 8 albino rabbits (4 of each sex), 2–3 kg in weight.
ii) Dose: Use 0.1 of the LD50 as determined in mice (as expressed in milligrams per kilogram).
iii) Day 0: Collect 10 ml of blood from each rabbit; separate serum from each sample and freeze. Clip the hair from the lateral abdominal area of each rabbit and give each of 3 males and 3 females intradermal injections of one dose of the drug; use multiple sites and inject no more than 0.2 ml in any one site. Into 1 male and 1 female rabbit (controls), inject a corresponding amount of vehicle in a similar manner.
Day 1: Examine the skin at the site of the day 0 injection.
Day 2: Examine the skin at the site of the day 0 injection; give the 6 experimental rabbits iv injections of an equal dose of the drug and the control rabbits the same volume of vehicle.
Days 4, 7, 9, 11, and 14: Give each experimental rabbit an iv injection of one dose of drug and the control rabbits one dose of vehicle.
Day 16: Collect 10 ml of blood from each rabbit; separate and freeze the serum. Repeat the skin test performed on day 0.
Days 17 and 18: Examine the skin sites for evidence of local reaction.
c) Capacity to produce antibodies in rabbits:
i) Determine passive hemagglutination titers according to the method of Stavitsky (*8*), with the modifications suggested by Peterson et al. (*9*).
ii) Use the frozen rabbit serum from the previous study.

SCHEDULING OF STUDIES

Because preclinical toxicity studies are usually the last ones completed before a drug is used in man, it is imperative that the studies be completed as rapidly as possible. If they are not completed on schedule, it often means the postponement of the initial clinical trials of a promising new antineoplastic agent. The identity and purity tests should be performed immediately upon receipt of the drug. The LD50 study in mice should be performed as soon as feasible. The single dose study in the dog should be started, with dose and route as specified by the project officer, as soon as dogs are available after preconditioning. When it is known that a drug will arrive from the NCI, preconditioned dogs should be available to start the study regardless of the completion of the identity and purity check.

Studies I, II, and III are open ended in the sense that doses ranging from the HNTD to the LD must be established. Therefore, whenever a toxic alteration is observed in one pair of treated animals, a dose at half the level can be started in another pair of animals. It is neither necessary nor is it preferable to wait until the extent of the toxicity is established or until the end of the study. Indeed, if toxicity is severe or lethality occurs, it is expedient to start two new pairs of animals at one-half and one-fourth the dose. If, on the other hand, little or no toxicity has appeared in a study in progress, start another two pairs of animals at twice and four times the dose. Studies IV and V are performed on dogs at dose levels defined in studies I and II so that escalation or reduction of doses is not required in these studies.

It is not desirable to perform the studies in numerical order. After the TDL is established in study I, proceed to study II. After both the TDL and TDH are established in study I, proceed directly to study V-A, V-B, or V-C. When the TDH is established in study II, start study III in monkeys. When the defined levels of toxicity are established in study II, begin study IV and, if requested, study V-D. Therefore, it is apparent that the simultaneous performance of several studies will be required.

For the purposes of the Laboratory of Toxicology, day 1 has arbitrarily been defined as the day of the first treatment and day −1 has been defined as the day preceding the day of the first treatment. In those instances when it is necessary to monitor a parameter of an animal on the same day as the first treatment but before the drug is administered, the date of such monitored parameters will be designated day 0.

PATHOLOGY PROTOCOL

The importance of proper examination and reporting of pathologic alterations in organs and tissues in a toxicologic study cannot be overemphasized. Precision and accuracy in reporting the type and severity of lesions in animals will increase the efficiency of prediction for toxic manifestations of antineoplastic agents administered to patients with neoplastic disease.

A. General Guidelines

In performing the necropsy and selecting tissues for histopathologic examination, the following guidelines should be observed:

1) Perform the necropsy on each animal as soon as possible after death or euthanasia in order to ensure the best possible conditions for pathologic examination.
2) If for some reason the animal cannot be necropsied immediately after death, place the animal in a refrigerator (4° C) (do not freeze) as soon as possible.
3) Perform each necropsy in a manner as near as possible to that described in this protocol. Whenever possible, the same person should conduct the necropsy and perform the histopathologic examination.
4) Examine all organs in situ before removal. Do not separate organs from connective tissue until the intervening tissue has been dissected and examined.
5) Record the gross observations on the keysort card provided.
6) Wash tissues with isotonic saline (0.85%), mammalian Ringer's solution, or Locke's solution, if required. Never use water on unfixed tissues that may be processed for histopathologic examination. The hypotonicity of water causes lysis of cells and induces artifacts within the tissues.
7) Incise each organ in order to: *a*) expose the greatest surface area, *b*) open structures that enter at the hilus, *c*) make visible the ductular and vascular structures, and *d*) preserve orientation and relations of the organ. All secondary incisions should be parallel to the first. When incising paired organs, make longitudinal incisions in the left and transverse incisions in the right organ to preserve their identities.
8) Obtain tissues for sectioning from the same specific site in a particular organ in each animal.
9) Obtain specimens of all grossly abnormal tissues for sectioning in addition to those routinely taken. Refer to table 2 for the list of tissues to be examined routinely.
10) Remove tissues for sectioning by cutting with a sharp knife or razor blade with quick, smooth, complete incisions to avoid compression. The use of scissors or dull knives results in undue pressure on the tissue and causes alteration of microscopic architecture.
11) Cut tissues obtained for histopathologic examination 0.5 cm in thickness or less to ensure rapid and complete fixation.
12) Store tissues in cold buffered neutral 10% formalin (BNF) prepared according to the formula in (*10*). Care should be taken in preparation and storage of BNF to maintain proper pH (7.0–7.4) to avoid deposition of acid hematin pigment in tissues.
13) Place tissues in a volume of BNF which is at least 15–20 times the volume displaced by the tissues. Rinse tissues in running tap water after 24–48 hours of fixation and place in fresh BNF.
14) Cover tissues that float in the fixative (e.g., lung tissue) with absorbent material (cheesecloth or gauze) in order to obtain universal penetration of the fixative.
15) Place tissue specimens that tend to curl in the fixative (e.g., skin tissue) on small pieces of 3 × 5 file cards prior to fixation.
16) Place all tissues, except the eyes, in BNF for at least 24, but not longer than 72, hours before further processing.
17) Refer to the Armed Forces Institute of Pathology Manual (*10*) for additional information on fixatives and special staining techniques.
18) Obtain samples for culture from eroded, ulcerated, and exudative lesions with sterile swabs. Routine bacteriologic culture of aseptically collected, postmortem heart blood should be performed with the blood of each animal.
19) Open covering (dura) of central nervous system (CNS) tissues (spinal cord and brain) to ensure proper fixation. Section CNS tissues for gross observation of internal structures after 72 hours of fixation in two changes of BNF.
20) Save representative tissue samples in a small amount of BNF in sealed plastic bags.
21) Record measurements of abnormal appearing organs or tissues in metric units (e.g., mm and mg). In general, the width, the greatest length, the greatest width, and the greatest depth should be recorded. On occasion special measurements may be required (e.g., thickness of heart wall, circumference of cardiac atrioventricular openings, and thickness of cortical and medullary areas of various organs).
22) Handle tissues with care in order to prevent induction of artifactual changes. Use proper equipment in removal of organs.
23) Save in a clean dry container all concretions and calculi for chemical analysis.
24) Measure (in ml), record specific gravity of, smear, and culture any excessive or abnormal body or joint fluids. Determine the character and type of cells present in the sediment.
25) Photograph significant gross and microscopic lesions. Refer to (*11*) for proper techniques used in gross medical photography.
26) Process bones, teeth, bone marrow, eyes, and CNS according to the special techniques listed in table 3.

B. Necropsy Protocol for Dogs and Monkeys

1) Perform a complete external examination and record the observations on the keysort card and on a form similar to form 4.
2) Examine and remove the eyes.
   a) Carefully examine the eyes of the animal.
   b) Removal of the eyes:
      i) Grasp the lids of one of the eyes at the lateral canthus with a toothed forceps.
      ii) Expose the orbit by incising through the skin and conjunctiva towards the medial canthus.
      iii) Grasp the lower bulbar conjunctiva and gently retract the globe downward.
      iv) Incise the conjunctiva above the globe by directing the scalpel into the orbit away from the globe.
      v) Enlarge the incision with the scalpel.
      vi) Insert a blunt-pointed curved scissors through the incision while exerting gentle traction downward with the forceps.
      vii) Direct the scissors upward and backward to the rear of the orbit and sever the optic nerve.
      viii) Cut the extraocular muscles with the scissors while exerting gentle traction on the conjunctiva with the forceps.
      ix) Remove the orbital contents in toto.
      x) Inspect the globe, optic nerve, extraocular mus-

cles, glands, third eyelid, and bulb for gross lesions.
xi) Dissect carefully the extraocular muscles away from the globe with the convex surface of the scissors.
xii) Measure the volume of the eye by placing it in a graduated cylinder containing fixative and recording the amount of fixative displaced.
xiii) Place the globe in Zenker's fixative.
xiv) Remove the other eye in the same manner.

3) Place the animal in dorsal recumbency.
4) Open the body cavities. All organs and structures that appear to be abnormal should be measured and weighed and the findings recorded on the keysort card and on a form similar to form 5.
a) Skin incision:
i) Begin incision in axillary region and continue along the midline in the following directions: *posteriorly* to the os pubis and *anteriorly* to the symphysis of the mandible.
ii) Divert the incision to the right of the prepuce in the male.
iii) Dissect the skin dorsally to both the right and left sides of the incision leaving the muscles in situ.
iv) Make transverse skin incisions at the os pubis and dissect the skin away from the midline.
v) Separate the front limbs from the thoracic wall at the scapula and lay dorsad.
vi) Disarticulate the coxofemoral joints by incising the muscles and ligaments in the area. Examine the coxofemoral joints.
b) Examine the prescapular and axillary lymph nodes.
c) Examine the mammary glands and inguinal and popliteal lymph nodes.
d) Expose the abdominal viscera and contents:
i) Make a small incision on the midline of the abdomen. Insert index and middle fingers through the incision; lift up and direct cutting edge of knife between fingers. Continue incision posteriorly along midline.
ii) Make transverse incisions of the abdominal wall posterior to the last rib and anterior to the os pubis.
iii) Reflect the abdominal wall to the right and left to expose the abdominal viscera.
iv) Examine the organs of the abdominal cavity in situ and note the appearance of the peritoneum and omentum, and the presence of fluids, hemorrhages, adhesions, or displacements. Measure and record on the keysort card the presence of abnormal body fluids.
e) Expose the thoracic viscera and contents: If pneumothorax is suspected, insert a 16-gauge needle attached to a 25-ml syringe filled with saline through an intercostal space. Bubbles appearing in the syringe denote air under pressure in the thorax.
i) Incise the diaphragm in an arc from one lateral side to the other lateral side.
ii) Remove the sternal plate by cutting each rib at both the right and left costochondral junction.
iii) Examine the organs of the thoracic cavity in situ noting the appearance of parietal and visceral pleura, and the presence of fluids, hemorrhages, adhesions, or displacements. Measure and record on the keysort card the presence of abnormal body fluids.
iv) Examine the pericardial cavity in situ for the quantity and consistency of fluids.
v) Remove heart blood in a sterile manner for culture.
f) Examine the right parotid, mandibular, and sublingual salivary glands and mandibular lymph nodes after dissection of the skin from the right side of the face.
g) Examine and remove the cervical lymph nodes and thyroparathyroid glands.

5) Remove and examine the thoracic viscera.
a) Removal of thoracic viscera:
i) Incise along the lingual surface of both sides of the ventral aspect of the mandible. Loosen the tongue, grasp it firmly, and pull in ventrally and posteriorly through the mandible while, at the same time, disarticulating the hyoid bones by transecting through the cartilaginous middle cornu with bone forceps. Incise the soft palate anterior to the tonsils so that the tonsils and adjacent pharynx will remain attached to the larynx and tongue.
ii) Grasp the tongue firmly and apply gentle tension by pulling with one hand while dissecting the trachea and esophagus from the muscles of the neck posteriorly towards the thoracic inlet.
iii) Continue the removal of the thoracic viscera by dissecting the aorta and mediastinum free from the dorsal thoracic wall.
iv) Sever the esophagus, aorta, and posterior vena cava just anterior to the diaphragm while elevating the trachea, esophagus, lungs, and heart. Cut the remainder of the mediastinum and pleural attachments to free the thoracic viscera.
v) Examine the parietal surfaces of the thoracic cavity along the ribs and diaphragm for adhesions, herniations, hemorrhages, or other lesions.
b) Examination of the oral cavity, thoracic viscera, and associated structures:
i) Examine the oral cavity carefully including the teeth, gingiva, labia, palate, and bucca.
ii) Examine the tongue.
iii) Examine the tonsils.
iv) Examine cervical and medical retropharyngeal lymph nodes.
v) Examine the esophagus for constrictions or sacculations before opening. Open and examine the mucosal surface and examine any contents which may be present.
vi) Examine the thymus and the bronchial and mediastinal lymph nodes.
vii) Examine and open the thoracic duct.
c) Examination of the respiratory system:
i) Examine the trachea and incise the larynx and dorsal portion of the trachea (tracheal muscle) exposing the mucosal surface.
ii) Incise the tracheal branches to expose the mucosa of the bronchi and some of the bronchioles. Observe for presence of exudate, hemorrhage, ingesta, or foreign bodies in the bronchial air passages.
iii) Examine the surface of the lungs for irregularities of color or texture.
iv) Palpate the lungs for irregularities of consistency (i.e., consolidation, atelectasis, emphysema, or fibrosis). Incise areas of firmness or softness by multiple parallel transverse cuts. Insufflate at least one lobe of the lung with buffered neutral formalin by gentle injection with a syringe. Tie off the hilus

of the lobe to prevent the escape of the fixative. Sections obtained for histopathologic evaluation should include the overlying pleura.

v) Examine the pulmonary arteries for thrombi, emboli, or other lesions by opening all major branches starting at least 2 cm above the pulmonary valves and continuing into the pulmonary parenchyma.

d) Examination of the heart and major vessels:

i) Observe and palpate the heart for any disproportion of parts (hypertrophy, dilatation, or anomalies).

ii) Examine the surface of the heart, especially the coronary vessels.

iii) Place the tongue with the trachea to the left and the apex of the heart toward the prosector. With the heart in this position, the pulmonary artery will be visible between the two auricles and the right ventricle will be facing up.

iv) Open the heart utilizing the preferred method of following the direction of blood flow.

a) Open inferior and superior vena cava.

b) Open right atrium and auricular appendage.

c) Open right ventricle by directing knife through the tricuspid valve and cutting through the right lateral border of the heart.

d) Open outflow tract of right ventricle by cutting parallel to septum and continuing through pulmonary valves into pulmonary artery.

e) Open left atrium and auricular appendage.

f) Open left ventricle by inserting knife through opening of mitral valve and incising along lateral border to the apex.

g) Open left ventricular outflow tract by cutting through the aortic leaflet of the mitral valve and into the aortic arch.

v) Examine the vessels, valves, and septa for lesions.

vi) Examine the endocardium and make multiple slices through the septum and ventricular walls to examine the myocardium and coronary vessels. Check the papillary muscles carefully for lesions.

vii) Open and examine the aorta and vena cava in their entirety.

viii) Wash heart free of blood and clots with saline. If the heart appears abnormal, remove it from the lungs and weigh it.

6) Remove and examine the abdominal viscera.

a) Removal of abdominal viscera:

i) Cut pubis and ischium on both right and left sides through obturator foramina.

ii) Remove the symphysis.

iii) Remove and examine the omentum.

iv) Examine spleen grossly and incise. Remove spleen.

v) Examine pancreas grossly and incise. Remove pancreas.

b) Examination and removal of the liver:

i) Examine the peritoneal surface for irregularities, adhesions, and other lesions.

ii) Free the liver from the diaphragmatic attachments.

iii) Free the liver from the mesentery.

iv) Transect the duodenum on each side of the entrance of the bile duct leaving a few centimeters of duodenum attached to the liver.

v) Cut post caval attachments.

vi) Remove the liver.

vii) Observe surfaces of all lobes noting size, shape, color, and consistency.

viii) Incise duodenum exposing mucosal surface in region of orifice of common bile duct.

ix) Squeeze gallbladder gently and observe for patency of common bile duct.

x) Open the gallbladder and note consistency of bile. Examine for stones, inflammation, thickening of wall, hemorrhages, and other lesions.

xi) Palpate and incise liberally all lobes of the liver. Observe for necrosis, fibrosis, abscesses, or other lesions.

c) Examination of adrenal glands in situ: Remove and section left adrenal longitudinally and right adrenal transversely.

d) Examination and removal of gastrointestinal tract:

i) Remove the stomach and all of the intestines except for the posterior rectum; do not sever.

ii) Free the intestines from the mesentery during removal and observe the mesenteric lymph nodes.

iii) Leave the mesenteric lymph nodes and mesentery attached near the ileocecal valve.

iv) Open the stomach along the greater curvature and observe the mucosal surface. Examine for hemorrhage, parasites, foreign bodies, abnormal ingesta, and other lesions.

v) Open the small intestine and observe all surfaces and ingesta.

vi) Open the cecum and colon posteriorly toward the anus and examine.

e) Examination and removal of genitourinary organs:

i) Remove kidneys, bladder, uterus, ovaries, vagina, vulva, rectum and anus of females as a unit. In males the prostate, and in monkeys the seminal vesicles, should be removed with the kidneys, bladder, rectum, and anus. The rectum is attached to the remaining intestinal mass and may be detached.

ii) Incise the left kidney in half longitudinally from the convex surface to the hilus and the right kidney transversely at the center.

iii) Remove the renal capsule from one half of each kidney and examine kidney surface. Note ease with which the capsule is removed. Make transverse incisions on the right kidney and longitudinal incisions on the left kidney. The tissue routinely saved for histopathologic examination from each kidney should be from the portions with the capsule intact.

iv) Open and examine the ureters, bladder, and urethra. Inspect mucosal and serosal surfaces and note patency of urinary channels.

v) Open vagina, cervix, and uterine horns along their dorsal borders and examine all surfaces.

vi) Examine ovaries for cysts, corpora, atrophy, and other lesions.

vii) Examine male accessory sex organs; note size, color, and consistency.

viii) Open inguinal canals following the spermatic cords to the testicles.

ix) Incise testicles and epididymides and examine.

x) Dissect prepuce and penis free from the abdominal wall and examine for lesions.

f) Examination of the abdominal aorta:

i) Dissect through the crura of the diaphragm and the subvertebral tissues to expose the abdominal aorta.

ii) Open the length of the aorta into the iliac branches

and carefully examine for thrombi, aneurysms, intimal plaques, and other lesions.
iii) Open the origins of the major branches of the aorta and examine the lesions as above.

7) Examine the musculoskeletal system.
a) Examination of the muscles:
i) Examine and incise the muscles of various parts of the body, especially the serratus ventralis, gracilis, longissimus dorsi, and the psoas muscles.
ii) Incise liberally the muscles of the abdominal and thoracic walls.
iii) Examine the muscles of the limbs and head.
b) Examination of the skeleton:
i) Isolate the right femur and make a transverse mid-shaft cut with a bone saw.
ii) Observe the thickness of the cortex.
iii) Examine the marrow and make several touch impressions of the marrow to glass slides.
iv) Collect sections of the costochondral junction and head of the right femur.
v) Examine the vertebral column and the vertebral discs for lesions.
vi) Incise the joints and examine for lesions.

8) Remove and examine the brain and spinal cord.
a) Removal of the brain:
i) Incise the ventral portion of the occipito-atlantal articulation and transect the exposed spinal cord.
ii) Decapitate at the occipito-atlantal articulation.
iii) Reflect the skin and muscles of head anteriorly and examine skull for lesions.
iv) Incise transversely with a saw over the dorsum of the skull posterior to the orbits.
v) Incise laterally with a saw each side of the cranium from the orbital fossa to the occipital condyles (leave room for brain to be removed intact).
vi) Remove cranial roof by elevating the anterior section and pulling dorsocaudad exposing the brain.
vii) Examine, incise, and remove dura mater from the dorsal part of the brain.
viii) Invert the skull allowing brain to descend out of the cranial cavity. Sever the cranial nerves with a scalpel to complete removal.
ix) Remove the pituitary gland by cutting diaphragmatic sella on both sides, clipping bony projection posterior to the gland, and cutting soft tissue around the gland proper with scissors.
b) Removal of spinal cord section:
i) Transect spinal column at L_2–L_3 and T_{11}–T_{12}.
ii) Remove spinal cord segment from spinal canal from the thoracolumbar region.
c) Removal of spinal cord in toto:
i) Remove skin, muscles, ribs, and limbs from the thorax.
ii) Place the spinal column and sacrum with the spinous processes of the vertebrae in an upright position.
iii) Dissect the muscles from the dorsum to expose the vertebrae.
iv) Cut through arches of vertebrae on each side of spinous processes with bone forceps or rachiotome saw and remove the bone to expose the spinal cord.
v) Cut through nerve roots on each side of cord and remove entire cord.
vi) Examine and incise dura mater prior to placing spinal cord in fixative.
d) Examination and removal of the sciatic nerve section:
i) Locate the sciatic nerve on the lateral aspect of the rear leg as it traverses the greater sciatic foramen (an opening in the pelvic wall bound ventrally by the os coxae and dorsally by the middle gluteal muscle).
ii) Remove a section of the sciatic nerve in an area of the largest diameter. Examine for evidence of gross lesions.

9) Examine cavities of the head.
a) Examination of the frontal sinus:
i) Cut transversely through frontal bones in midorbital region with bone saw to expose frontal sinuses if not already visible after removal of the brain.
ii) Examine orbital portion of the sinuses.
iii) Examine nasal parts of the sinuses.
b) Examination of the maxillary sinus:
i) Cut transversely through maxillary bones in the region of the first molar tooth.
ii) Examine maxillary sinus.
iii) Examine nasomaxillary opening.
c) Examination of the nasal sinus:
i) Cut transversely through nasal bones immediately posterior to the canine tooth.
ii) Examine nasal cavity for asymmetry and lesions.
iii) Examine septum for asymmetry and lesions.
iv) Examine the three paired nasoturbinate bones for asymmetry and lesions.
d) Examination of the tympanic cavity:
i) Cut transversely through the external auditory meatus exposing the tympanic cavity proper.
ii) Examine tympanic fundus by sawing or breaking through the ventral aspects of the tympanic bulla.

C. Systematized Nomenclature of Pathology (SNOP)

1) The SNOP system is a descriptor-type information retrieval system for handling medical language data (*13*). It is a systematized nomenclature and not a standardized language nor a standard coding system. Developed and written specifically to assist pathologists in organizing and utilizing material derived from pathologic evaluation of human subjects, the system has been used in processing animal data. Whenever a contractor's pathologist discovers a discrepancy or a missing term as the SNOP system applies to the evaluation of canine and simian tissues, the project officer should be notified in writing so that future modifications can be instituted. The SNOP system is divided into four highly structured semantic categories as follows:

Topography (TOPO): the body site affected;
Morphology (MORP): the structural changes or lesions;
Etiology (ETIO): the causative agent(s);
Function (FUNC): the manifestations associated with a pathologic condition, including signs and symptoms and a number of specific infectious diseases.

In addition to the use of the SNOP system, the contractor's pathologist is requested to grade the lesions observed in the dogs and monkeys according to degrees of severity with Roman numerals I through IV. Changes in clinical parameters are to be rated with Arabic numbers 1 through 9 (table 3). A keysort punch card is provided to contractors for the pathology report on each animal. Upon termination of preclinical toxicologic studies of an anticancer agent, the completed keysort cards are to be

returned to the Laboratory of Toxicology with the toxicology report.

2) Directions for reporting on keysort card.
   a) Front side (text-fig. 1)
   i) *Upper portion:* Most of the information requested is self-explanatory. The length of the animal should be an overall measurement from the top of the skull to the base of the tail. Days on study should be the total number of days elapsed from administration of the first dose of the drug to the day of necropsy. If a dead animal is placed in a refrigerator before the necropsy, record the number of hours refrigerated. All information should be filled in except for the "Drug Class," which will be completed by the Laboratory of Toxicology.
   ii) *Lower portion:* To complete this section, utilize the SNOP system (*13*).[3] Enter all abnormal findings by locating the appropriate four-digit code in the SNOP lexicon and filling in the correct code under the specific category column. The majority of findings will result in utilization of categories TOPO and MORP. Whenever the etiology is known or a dysfunction is observed, use appropriate codes for categories ETIO and FUNC, respectively. If the lesion is evident grossly, check the proper square under column "g," and, likewise, if evident microscopically, check the appropriate square under "m." Finally, rate the severity of the lesion by placing the proper Roman numeral under column "R."
   b) Reverse side (text-fig. 2)
   i) *Upper portion:*
      a) *Clinical summary:* Estimate the *overall* alteration of each clinical parameter, and rate the severity of these changes utilizing the one-digit Arabic code 1–9 (table 4).
      b) *Specialized techniques:* Check appropriate square if photographs are taken. List microorganisms identified from heart blood culture. If special fixatives and/or stains are used in the histopathologic evaluation, list them in the space provided.
      c) *Abnormal body fluids:* Enter quantity (ml) of abnormal fluids in appropriate blanks.
      d) *Abnormal tissues:* Measure and weigh abnormal organs and tissues. Use the four-digit code (TOPO) designation to identify the structures involved.
   ii) *Lower portion:* Summarize the pathologic findings correlating the structural alterations with changes in clinical parameters. Emphasize those changes which appear to be drug related and differentiate them from the alterations associated with other etiologic factors (e.g., parasitic, traumatic, nutritional). Enter results of special clinicopathologic tests such as EEG's and ECG's in this section.

REPORTING PROCEDURES

A. Reports must be typed clearly and submitted on 8½ × 11-inch bond paper.

B. Fifty copies of each report are required and each will include:
   1) A coversheet as required by the Department of Commerce Clearinghouse (provided by the Laboratory of Toxicology).
   2) Contract number, drug NSC number and name, date received, doses expressed as milligrams/kilogram and milligrams/square meter, control values as established by the contracting laboratory, methodology or references for individual test procedures, and strains and sources of all experimental animals.
   3) Chemistry information sheet: chemical data, lot or batch number, and results of the identity and purity tests.
   4) Summary of observations in prescribed formats.
   5) Overall evaluation and clinical pathogenesis of toxicologic features of the drug written in a format suitable for publication in Part 3 of *Cancer Chemotherapy Reports* or other recognized scientific journals.
   6) Attestation by and signature of principal investigator.
   7) A complete pathology report on each test animal must be submitted on the supplied keysort cards. The original card on each animal on test must be returned with the final report.

C. The final report is due 60 days after the last animal died or was killed.

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[3] The SNOP lexicon can be purchased from the College of American Pathologists, 230 North Michigan Ave., Chicago, Ill. 60601.

TABLE 1.—*Dog and monkey studies of the Laboratory of Toxicology*

Study	Dose levels[a]	Number of animals[b]	Days of treatment	Day of killing	Days of post-treatment observation
I (dogs)	1 HNTD	2	1	8, 46	7, 45
	2 TDL	2	1	8, 46	7, 45
	4 TDH	2	1	8, 46	7, 45
	8 LD	2	1	8, 46	7, 45
II (dogs)	1 HNTD	2	1-5	6, 50	1, 45
	2 TDL	2	1-5	6, 50	1, 45
	4 TDH	2	1-5	6, 50	1, 45
	8 LD	2	1-5	6, 50	1, 45
	Untreated controls	2	—	6, 50	—
III (monkeys)	1 HNTD	2	1-5	6, 50	1, 45
	2 TDL	2	1-5	6, 50	1, 45
	4 TDH	2	1-5	6, 50	1, 45
	8 LD	2	1-5	6, 50	1, 45
	Untreated controls	2	—	6, 50	—
IV (dogs)	1 HNTD (from study II)	2	1-5, 15-19, 29-33	34, 78	1, 45
	2 TDL (from study II)	2	1-5, 15-19, 29-33	34, 78	1, 45
	4 TDH (from study II)	2	1-5, 15-19, 29-33	34, 78	1, 45
V-A (dogs)	2 TDL (from study I)	2	1, 8, 15, 22, 29, 36	39, 83	1, 45
	4 TDH (from study I)	2	1, 8, 15, 22, 29, 36	39, 83	1, 45
V-B (dogs)	2 TDL (from study I)	2	1, 8, 15, 22, 29, 36	39, 83	1, 45
	4 TDH (from study I)	2	1, 8, 15, 22, 29, 36	39, 83	1, 45
V-C (dogs)	2 TDL (from study I)	2	1, 8, 15, 22, 29, 36	37, 81	1, 45
	4 TDH (from study I)	2	1, 8, 15, 22, 29, 36	37, 81	1, 45
V-D (dogs)	1 ½HNTD (from study II)	2	1-10	11, 55	1, 45
	2 ½TDL (from study II)	2	1-10	11, 55	1, 45
	4 ½TDH (from study II)	2	1-10	11, 55	1, 45
	8 ½LD (from study II)	2	1-10	11, 55	1, 45

[a] *Numbers* indicate geometric escalation of doses (1, 2, 4, 8, etc).
[b] One male and 1 female in each pair.

TABLE 2.—*Tissues to be processed for histologic sectioning and staining*

System	Tissues
Cardiovascular	Heart Left ventricle and septum Right ventricle Abdominal aorta
Digestive	Lip Tongue Salivary gland (parotid) Esophagus (thoracic) Stomach (fundic area) Duodenum (region of common bile duct and (pancreatic duct) Jejunum Colon Liver Right medial lobe with section of gall bladder Left lateral lobe
Pancreas	Head Tail
Endocrine	Adrenal Parathyroid Pituitary Thyroid
Hematopoietic	Bone marrow (femur): section plus touch impressions Spleen
Integumentary	Site 1: nonfriction surface—dorsal thorax Site 2: friction surface—elbow Injection site Mammary gland, both sexes (left cranial abdominal in dogs and left in monkeys)
Skeletal	Rib (costochondral junction of 6th right rib) Femur (head)
Lymphatic	Thymus Lymph nodes Bronchial Mesenteric Mandibular Regional in area of injection site Tonsil (palatine)
Muscular (diaphragm)	
Nervous	Brain Site 1: cerebral cortex (left lateral gyrus) Site 2: cerebellum Site 3: medulla (posterior to pons) Spinal cord (thoracolumbar region) Peripheral nerve, sciatic (mid-femur region)
Reproductive	Male Left testicle and epididymis Prostate Seminal vesicle (monkey) Female Left ovary Uterus (body)
Respiratory	Trachea Lung Left diaphragmatic lobe Left apical lobe
Special senses	Eye (right and left) Ear (left external auditory meatus)
Urinary	Kidneys Right transverse section Left longitudinal section Ureter (left) Bladder (fundus)

TABLE 3.—*Tissues requiring special handling*

Tissues	Procedure
Bones and teeth	These structures should be decalcified before sectioning if a bone sectioning machine is not available. Several acceptable methods for sectioning undecalcified and decalcified tissues are listed in (*10*). Prior to fixation, tissues should be cut with a fine saw into thin pieces, approximately 5 mm in width.
Bone marrow	Several touch impression smears should be made of the femoral and costal bone marrow. The smears should be fixed in methanol for 3–5 minutes, rapidly air dried, and stained by the May-Grunwald-Giesma technique or another suitable method (*10*).
Eyes	The eyes should be removed quickly from the animal according to the method of Saunders and Jubb (*12*) and put in Zenker's fixative for 8 hours. After fixation, the eyes should be washed in cold running tap water for 12 hours and stored in 70% alcohol until ready for further processing. Prior to embedding, they should be hardened by dehydration in graded alcohols. Twenty-four hours each in 70, 85, and 95% alcohol is usually sufficient for dehydration. Parasagittal calottes should be removed from each side of the eye after hardening.
Brain and spinal cord	The whole brain and a segment of spinal cord should be routinely removed, examined, and fixed. When a neurologic disturbance is suspected, the whole spinal cord should be removed. After removal of the dura mater, the whole brain and the spinal cord segment should be fixed in BNF for 24–48 hours. After initial fixation, tissues should be examined carefully for lesions and 0.5-cm sections taken for processing. These sections must be fixed in BNF for another 24 hours before dehydrating and embedding.

TABLE 4.—*SNOP rating codes*

Lesions	Definitions	Clinical parameters [a]		Definitions
I	Minimal: least possible degree (+), 10% of area affected	Below normal	1	0–0.25 times normal
II	Moderate: mild (++), 10–50% of area affected		2	0.26–0.50 times normal
III	Marked: severe (+++), 50% of area affected but less than total		3	0.51–0.75 times normal
IV	Maximal: greatest possible degree (++++), massive, total, extreme		4	0.76 times normal to low normal range
		In normal range	5	
		Above normal	6	In high normal range to 1.5 times normal
			7	1.6–2.0 times normal
			8	2.1–2.5 times normal
			9	2.6 times normal and greater

[a] Keysort designation.

FORM 1.-*Urinalysis*

NSC—	Dog or monkey No.:	mg/kg:
Dose formulation:	Route and regimen:	mg/m²:

Urinalysis	Day													
Specific gravity														
Sugar														
Protein														
Ketones														
Bilirubin														
Color														
Microscopic														
RBC/HPF														
WBC/HPF														
Casts/LPF														
Epithelial cells														
Crystals														
Bacteria														

FORM 2.—*Hematologic and blood chemistry tests*

NSC—	Dog or monkey No.:	mg/kg:
Dose formulation:	Route and regimen:	mg/m²:

		Day												
Hematologic tests														
Hematocrit (PCV)	(a)													
Hemoglobin	( )													
RBC	( )													
Platelets	( )													
Reticulocytes	( )													
WBC	( )													
Neutrophils, immature	( )													
Neutrophils, mature	( )													
Lymphocytes	( )													
Monocytes	( )													
Eosinophils	( )													
Basophils	( )													
Blood chemistry tests														
BUN	( )													
Prothrombin time	( )													
Blood sugar	( )													
Serum creatinine	( )													
SGOT	( )													
SGPT	( )													
Bilirubin, total	( )													
Bilirubin, direct	( )													
BSP, 15 min	( )													
Alkaline phosphatase	( )													
Na	( )													
K	( )													
Ca	( )													
Cl	( )													
Mg	( )													

[a] Normal range as established in contracting laboratory; include unit of measurement.

FORM 3.—*Record of observations*

NSC—
Dose formulation:

Dog or monkey No.:
Source: mg/kg:
Route and regimen: mg/m²:

Dosage protocol						Toxicity sequence
Day	Daily dose		Cumulative dose		Weight, kg	Observations
	mg/kg	mg/m²	mg/kg	mg/m²		
						Cause of death:

FORM 4.—*Report of pathologic changes*

NSC—
Dose formulation:

Dog or monkey No.: mg/kg:
Route and regimen: mg/m²:

☐ Died ☐ Killed ☐ Moribund ☐ Day ____

Method of euthanasia:

Tissues Examined

Tissue	Observations	
	Gross pathologic changes	Histopathologic changes
≈		
≈		

FORM 5.—*Organ weights*

NSC—
Compound name:
Animal No. and Sex:
Dose { mg/kg/day: / mg/m²/day:
No. of treatments:
Body wt (kg) { Start: / Final:

Organ	Weight
Ovaries	
Pituitary	
Testes	
Thyroid and parathyroids	
Adrenals	
[a]	
[a]	
[a]	
[a]	

[a] Record weight of all organs that appear abnormal grossly in addition to those indicated.

FORM 6.—*Summary of mouse single-dose toxicity data*

NSC—
Compound name:

Strain of mouse:

	Male			Female		
Body wt — Start:						
Body wt — Final:						
Formulation:						
Route of administration:						
Dose and proportion of deaths	Dose mg/kg	No. of deaths	Dose mg/m²	Dose mg/kg	No. of deaths	Dose mg/m²

Dose and proportion of deaths	Male			Female		
	Dose, mg/kg	No. of deaths	Dose, mg/m²	Dose, mg/kg	No. of deaths	Dose, mg/m²
LD90						
(95% confidence limits)						
LD50						
(95% confidence limits)						
LD10						
(95% confidence limits)						
Probit mortality/log dose slope						
(95% confidence limits)						

FORM 7.—*Compatibility testing of dosage formulation*

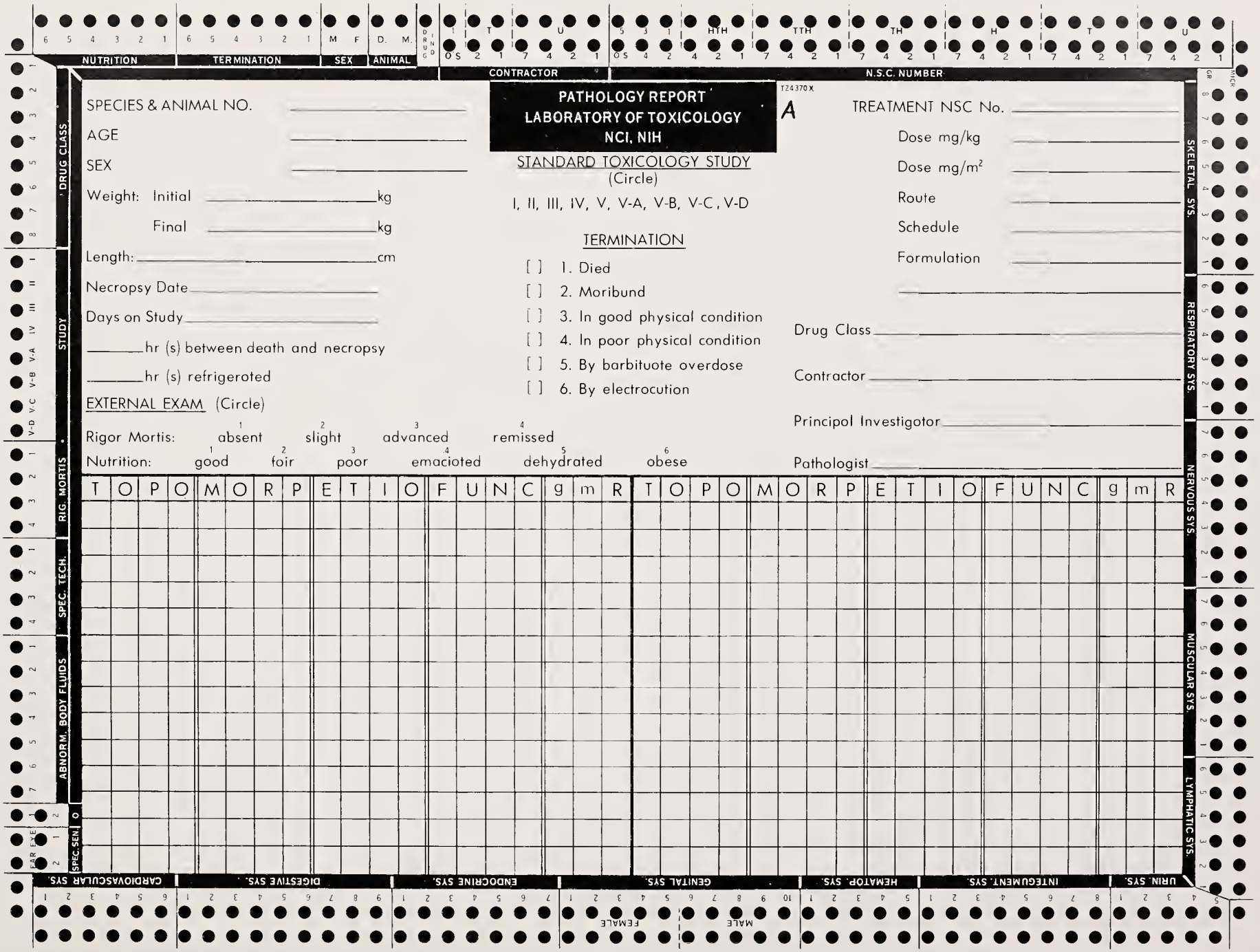
Ratio of drug or vehicle to serum, plasma, or blood	Serum [a]		Plasma [a]		Blood [b]			
	Drug	Vehicle	Drug	Vehicle	Drug	Vehicle	Control	Saponin control
1:1								
1/2:1								
1/4:1								
1/8:1								
1/10:1								
1/32:1								

[a] Expressed as compatible or incompatible.
[b] Expressed as grams of hemoglobin/100 ml of plasma.

FORM 8.—*Summary of local action and irritating effects of drug in rabbits and guinea pigs*

NSC—
Formulation:

Test animal	Concentration	pH	Volume injected, ml	Histopathologic changes



TEXT-FIGURE 1.—Front side of keysort card for pathology report for Laboratory of Toxicology, NCI, NIH.

CLINICAL SUMMARY

Check

[ ] weight loss
[ ] anorexio
[ ] diorrheo
[ ] emesis
[ ] adipsio
[ ] polydipsia
[ ] onuria
[ ] polyuria

1-9 Rating

Blood & Serum

[ ] Hemotocrit
[ ] Hemoglobin
[ ] RBC
[ ] Platelets
[ ] Reticulo.
[ ] WBC

[ ] Neutro.
[ ] Moture
[ ] Immature
[ ] Eosin.
[ ] Lympho.
[ ] Mono.
[ ] BUN
[ ] Proth. time
[ ] Blood sugar
[ ] Serum creat.
[ ] SGOT
[ ] SGPT
[ ] Bilirubin
[ ] Total
[ ] Direct
[ ] BSP
[ ] Alk. Phos.

B

[ ] Na
[ ] K
[ ] Ca
[ ] Cl
[ ] Mg

Urine

[ ] Sp. gr.
[ ] Sugar
[ ] Protein
[ ] Ketones
[ ] Bilirubin
[ ] RBC
[ ] WBC
[ ] Costs
[ ] Crystals

SPECIALIZED TECHNIQUES

[ ] Gross Photographs Blood Culture _____
[ ] Photomicrographs _____
Special Fixatives _____
_____
Special Stains _____
_____

ABNORMAL BODY FLUIDS (ml)

_____ 1 Ascitic
_____ 2 Cerebrospinol
_____ 3 Cystic
_____ 4 Edemo
_____ 5 Synoviol
_____ 6 Thoracic
_____ 7 _____

ABNORMAL TISSUES

M	O	R	Y	WT. (mg)	SIZE (mm)	
					X	X
					X	X
					X	X
					X	X
					X	X
					X	X
					X	X
					X	X

V23206X

CLINICOPATHOLOGIC SUMMARY

Text-figure 2.—Reverse side of keysort card.

Part II

Brief Historical Statement: Chemotherapy of Malignant Tumors in the U.S.S.R.

N. I. Perevodchikova[1]

The development of cancer chemotherapy in the Soviet Union is closely related to that of cancer research and cancer control. The public health system was organized even as the Soviet Republic came into existence. Cancer control, an essential part of that system of public health, shares in the benefits common in the Soviet public health system: completely free medical care, universal affability, prophylaxis, dispenserization, and social care in cases of partial or complete invalidism.

The modern system of cancer control was organized in 1945. At present, the U.S.S.R. oncologic network includes 22 research institutes, the largest of which is the Institute of Experimental and Clinical Oncology,[2] U.S.S.R. Academy of Medical Sciences (Moscow), that coordinates research in experimental oncology, cancer chemotherapy, radiation therapy, and cancer epidemiology. Others include the N. N. Petrov Oncological Institute of the U.S.S.R. Ministry of Public Health (Leningrad), the P. A. Hertzen Oncological Institute in Moscow (heading cancer control in R.S.F.S.R.), Institute of Problems of Oncology of the Ukrainian Academy of Sciences (Kiev), and a number of institutions of oncology and radiology of the Ministries of Health of the Republics of the Soviet Union.

The basic unit in the U.S.S.R. cancer control system is the oncologic dispensary, which is an organizational, methodological, and clinical center for the territory of a republic, region, city, and district. As a rule, it has a hospital with all the modern equipment necessary for cancer treatment and a registry for the continuous observation of cancer patients; it is responsible for the diagnosis of new cancer cases. At present, we have 253 oncologic dispensaries and 3,176 oncologic departments and offices.

Special medical education for oncologists is provided through aspirantur[3] and ordinatur (postgraduate work),[4] as well as at the chairs of oncology at the institutes and dispensaries. Chairs of oncology have recently been established in a number of medical schools.

Currently, several large cancer centers are under construction; the largest among them is the All-Union Oncological Center of the U.S.S.R. Academy of Medical Sciences in Moscow.

Coordination of all oncologic research is the responsibility of the Scientific Council on the Problem of Malignant Neoplasms of the Presidium of the U.S.S.R. Academy of Medical Sciences. The members of this Council are the most prominent oncologists working at institutions that are under the Ministry of Public Health and at other medical facilities. Ten "problem commissions" work with the Council. The main tasks of the Council and its 10 problem commissions are to choose the principal trends of investigation and to coordinate the activity of all the institutions engaged in cancer research.

Chemotherapy of tumor diseases and its associated problems have been extensively studied in the Soviet Union since 1950. The first workers in this field were L. F. Larionov and his colleagues, who searched for antitumor compounds among the alkylating agents, in particular, the chloroethylamine derivatives. This work was started in the Leningrad Institute of Oncology, U.S.S.R. Ministry of Public Health, in cooperation with a number of Leningrad chemists. After the Institute of Experimental and Clinical Oncology (IECO), U.S.S.R. Academy of Medical Sciences, was organized in 1952, the research was continued in Moscow.

One of the principal areas of scientific investigation at IECO was chemotherapy of tumor diseases. The intensive work in experimental and clinical chemotherapy was directed by N. N. Blokhin. A commission on the problem of chemotherapy of tumor diseases is now coordinating scientific research on this aspect.

The research of L. F. Larionov and his co-workers led to the creation of novembichin (1950); although rarely used now, it was quite popular in its time. Dopan (1955) occupied a definite place in the treatment of lymphogranulomatosis (Hodgkin's disease). The widely known sarcolysin was also developed by them in 1954.

In the last years of his life, L. F. Larionov concentrated his efforts on the study of the antitumor properties of the sarcolysin peptides (L. F. Larionov, 1957, 1961; L. F. Larionov and Z. P. Sof'ina, 1957). In collaboration with chemists of the school of I. L. Knuniants and others, the sarcolysin peptides and a number of amino acids, valine (asaline), leucine (asaley), tyrosine (astyran), methionine (asamet), etc., were synthesized and studied.

Under the supervision of V. A. Chernov, a group of investigators, working in the Ordzhonikidze All-Union Scientific Research Chemical–Pharmaceutical Institute (also mainly studying alkylating compounds), developed original compounds of the ethyleneimine group: dipine (1957) and thiodipine (1962). Among the chloroethylamines transferred to clinical practice by these investigators, prospidine, with its low suppressive effect on hematopoiesis, should be mentioned.

Ethyleneimine derivatives are being studied actively at the Kiev Sanitary–Chemical Institute, Ukrainian S.S.R. Ministry of Public Health, where such preparations as fluorobenzo-TEPA, iodobenzo-TEPA, and diiodobenzo-TEPA were developed.

Under the direction of N. M. Emanuel (Institute of Chemical Physics, U.S.S.R. Academy of Sciences), the study of antitumor compounds of the radical reaction inhibitor type was begun in the 1960's, and of nitrosourea derivatives in the second half of that decade. This work was done with the active

[1] Chief, Department of Experimental Chemotherapy, Cancer Research Center, Academy of Medical Sciences, Moscow, U.S.S.R.

[2] Since this monograph has gone to press, the name of this Institute was changed. It is now known as the Cancer Research Center.

[3] Aspirant for the degree of Candidate of Medical Sciences.

[4] Physician who has attained specialization in oncology and may be a Candidate or Doctor of Medical Sciences.

participation of the late E. M. Vermel, and as a result of these investigations, methylnitrosourea was made available for clinical use.

The search for new antitumor compounds in the antimetabolite group began later in the Soviet Union than did the research of alkylating agents. Among the original Soviet compounds, the creation of ftorafur in 1967, under the leadership of S. A. Hiller (Institute of Organic Synthesis, Latvian Academy of Sciences), attracted the greatest interest. It is used in the U.S.S.R. primarily for breast, colon, and rectal cancers.

Active research for new antitumor antibiotics, begun by M. M. Maevskii, at IECO, is presently concentrated in the Institute for the Search for New Antibiotics, U.S.S.R. Academy of Medical Sciences, where, under the leadership of G. F. Gauze, olivomycin, rubomycin, bruneomycin, and carminomycin have been produced.

Finally, although study of antitumor compounds of plant origin was until recently performed on a small scale, the work of G. P. Men'shikov (the first to isolate and investigate colchamines) must be mentioned.

In the paper devoted to trends in research for new antitumor compounds, M. N. Preobrazhenskaya discussed in detail the work being done in the development of new antitumor compounds in various institutions. Approximately 800 to 1,000 new antitumor substances of synthetic and natural origin are developed for experimentation each year. A general feature of these studies is the directed search for new compounds.

Of great importance for broadening the scope of cancer chemotherapy research was the organization of specialized chemotherapeutic clinical departments. The first such department was established in IECO in 1960 and was headed by V. I. Astrakhan. Such departments now exist in most of the oncologic institutes of our country, as well as in a number of large dispensaries specializing in tumor therapy.

In 1966, the All-Union Antitumor Chemotherapeutic Center (first director, A. M. Garin) was established in IECO; its mission was to organize cooperative clinical testing of antitumor compounds in 120 clinical institutions. That same year, a unified method for clinical testing of new antitumor compounds and unified criteria for evaluating their effectiveness were first adopted; both were approved by the Pharmacological Committee, U.S.S.R. Ministry of Public Health.

Results of the latest clinical research in methodology are presented in the section "Clinical Test Methods for Antitumor Drugs in the U.S.S.R.," by A. M. Garin and M. R. Lichinitser. The Pharmacological Committee is the only organization in our country authorized to approve clinical tests of new drugs and to certify their widespread clinical use. In conformance with this, experimental data on a new compound having antitumor activity are sent to this Committee, which examines the data and, after approving the tests, sends all the information to the All-Union Antitumor Chemotherapeutic Center, where specialists perform the tests and devise clinical studies for the use of the respective drug.

The first phase of clinical tests, in accordance with the order of the Ministry of Public Health, can only be performed in two specialized oncologic institutions: at IECO or at the Institute of Oncology, Ministry of Public Health (Leningrad). The second phase is organized by the All-Union Chemotherapeutic Center in several institutions, so that a sufficient number of observations can be made in a comparatively short time and a sufficient number of patients with each "signal" tumor type can be treated.

This system of organization of cooperative tests reduces the time for clinical testing of new compounds to about 2 years, on the average.

After the results have been examined, the Pharmacological Committee decides on the possible use of the new antitumor compound in clinical practice.

A number of domestic antitumor compounds are now being used in Soviet oncologic institutions. A survey of them is presented in V. I. Borisov's paper "Soviet Antitumor Preparations."

The Mechanisms of Action of Antitumor Compounds

A. K. Belousova[1]

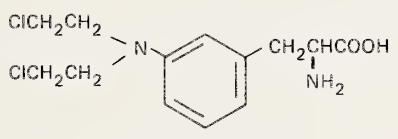
The study on the molecular mechanisms of action of antitumor compounds is a basis for the rationale of cancer chemotherapy. First, determination of the structure–activity relationships in a series of antitumor compounds provides a basis for the synthesis of new, more perfect inhibitors of essential tumor-cell enzymes. Second, knowledge of the molecular mechanisms of action and pharmacokinetics of various groups of antitumor compounds makes it possible to develop rational schemes for their clinical use.

Three classes of antitumor compounds are widely used at present in oncologic practice: alkylating agents, antimetabolites, and substances of natural origin (antibiotics, plant alkaloids, hormones). In this paper, data on the mechanisms of action of the first two groups of antitumor compounds are considered.

ALKYLATING AGENTS

Nitrogen Mustards

The first antitumor compounds in this group [embichin (HN2), novembichin] had extremely low selectivity and limited activity on human tumors (*70, 72*). In searching for new and more effective antitumor compounds, Soviet and foreign scientists successfully use the principle of joining cytotoxic groups to different types of carriers (*69–74, 148*). L. F. Larionov's idea (*70, 71*), on the use of essential metabolites (amino acids, peptides, pyrimidines, purines, vitamins, hormones) as carriers of alkylating "warheads," led to the synthesis of a number of valuable compounds with high selectivity of antitumor action. This investigation was highly fruitful in the Soviet Union (*28, 72, 74, 75, 111, 125, 139, 141*). Sarcolysin or melphalan (*p*-di(2-chloroethyl)amino-L-phenylalanine) occupies a special position among this type of "alkylating metabolites" (*75*).



Sarcolysin (melphalan)

This was the first of the chloroethylamines, known at that time, which caused complete remission of a number of experimental tumors after a single administration to rats at the maximum tolerable dose. Only moderate toxic effects on normal tissues were observed (*71, 75*).

The cause of the high selectivity of the antitumor effect of sarcolysin was sought first and foremost in the natural carrier, phenylalanine. It was proposed that sarcolysin possessed both alkylating and antimetabolite properties. This idea appeared to be confirmed by differences in antitumor effects and inhibition of DNA synthesis by the L and D isomers of sarcolysin (*90, 132*).

L. F. Larionov suggested that the L-sarcolysin molecules may be incorporated in the proteins of tumor cells by peptide bond formation; however, experiments did not confirm this. In in vitro experiments with sarcolysin labeled in the β-carbon of alanine, it was shown that, in tumor cells, the drug binds much more extensively with nucleic acids than with proteins. Binding is not inhibited by either an excess of phenylalanine or by a high concentration of the protein synthesis inhibitor chloramphenicol. Hydrolyzed preparations of ^{14}C-sarcolysin, devoid of alkylating capacity, do not bind to tumor cell nucleic acids and proteins (*49*). These data strongly suggest that the interaction of sarcolysin with tumor-cell nucleic acids and proteins takes place only by alkylating reactions.

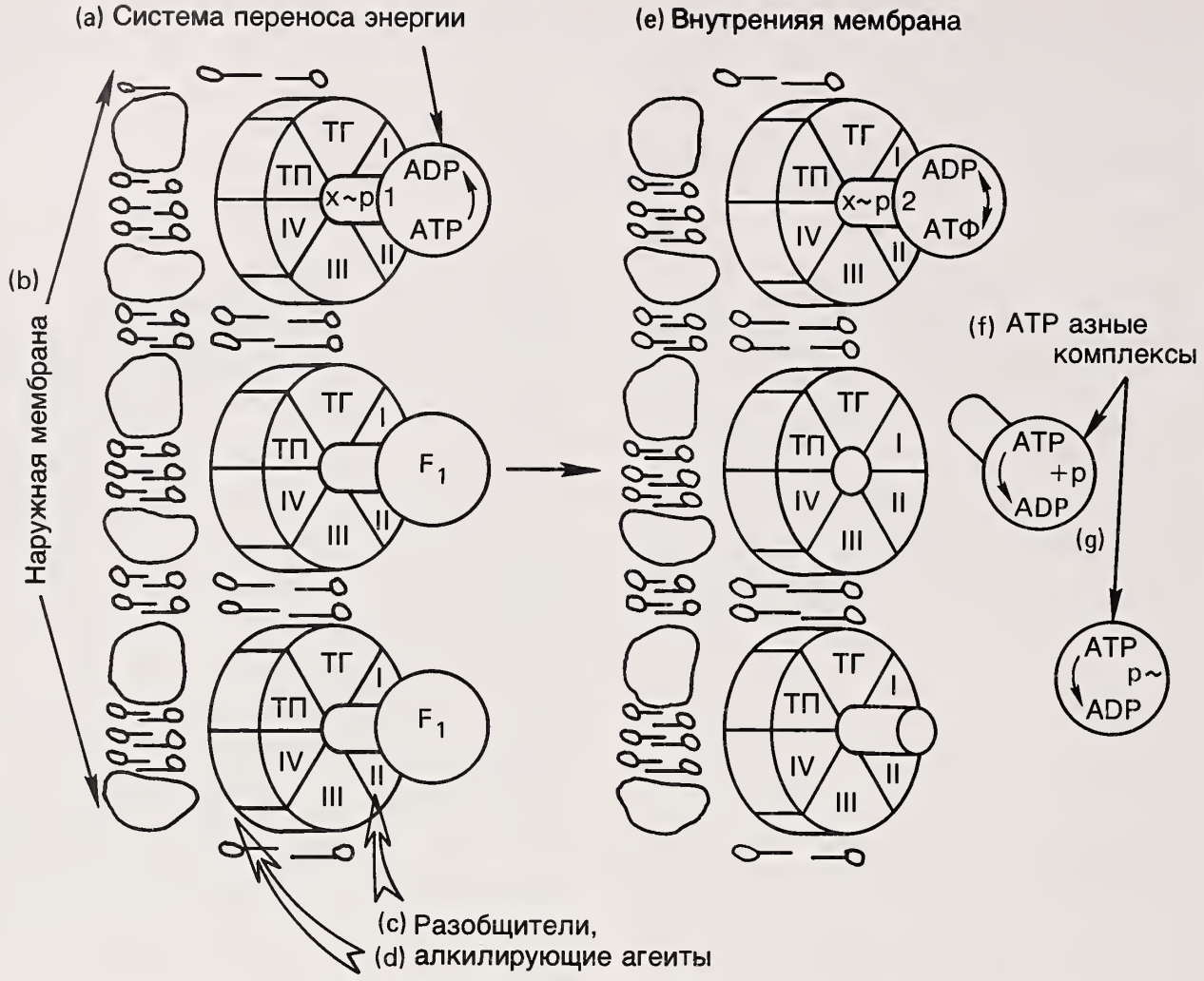
Several researchers (*9, 37, 67, 80, 88, 91, 106, 147*) established that, like other chloroethylamines, sarcolysin alkylates nucleic acids and proteins of normal tissues and tumors, induces formation of cross-links and breaks in DNA molecules and cross-links between DNA and chromatin proteins, and selectively alkylates histones. Alkylation of DNA and nuclear protein results in disturbances in DNA replication and transcription and mutagenic effects (*41, 65, 66, 89*).

At the same time, in contrast to HN2 and other chloroethylamines, sarcolysin in therapeutic doses inhibits respiration and oxidative phosphorylation in cells of sarcoma 45 but not in cells of a drug-resistant subline of this tumor (*38, 63*). Active amino acid transport in tumor cells is also suppressed by sarcolysin (*93*).

When incubated with ascites tumor cell suspensions, sarcolysin (1 mmole) causes complete distribution of their energetics, as evidenced from the rapid degradation of ATP and ADP and accumulation of AMP (*102*). These observations suggest that sarcolysin may have cytotoxic effects on the mitochondria of tumor cells.

As is well known, the mitochondria perform the function of principal energy generator in normal and tumor cells (*7*). A respiratory enzyme system and oxidative phosphorylation components are built into the inner membrane of the mitochondria (text-fig. 1). Coupling of electron transport and energy transformation is localized in headpieces of the inner membrane, in the heads of which are located the enzymes accomplishing ATP synthesis (*120, 149*). In intact mitochondria, the reaction is shifted sharply in the direction of ATP synthesis; however, under the action of uncouplers, the coupling mechanism is disrupted and the enzyme begins to function as ATPase. Activation of latent ATPase frequently is

[1] Head, Laboratory of Biochemical Pharmacology, Cancer Research Center, Moscow, U.S.S.R.



TEXT-FIGURE 1.—Diagram of mitochondrial membrane as an association of supramolecule complexes accomplishing electron and energy transport (according to Green): I, II, III, IV=electron transport complexes; ТГ (TH)=transhydrogenase; ТЛ (TP)=transprotonase; *arrows* indicate sites of action of uncouplers and alkylating compounds causing a breaking away of ATPase complex F_1 from the inner membrane. 1=ADP; 2=ATP; a=energy transport system; b=outer membrane; c=uncouplers; d=alkylating agents; e=inner membrane; f=ATPase complexes; g=phosphate; X≈P=high energy phosphate.

accompanied by a breaking away of the heads from the inner membrane of the mitochondria.

The effect of sarcolysin on the oxidative phosphorylation reactions in mitochondria of normal and tumor cells is similar to that of classic uncouplers of the dinitrophenol type. It uncouples respiration and phosphorylation at almost every point (*7, 14*). The uncoupling effect, probably localized at the level of the unphosphorylated high-energy intermediate X~P, is manifested by the loss of respiratory control, a blockage of ATP synthesis, and activation of latent mitochondrial ATPase, and then is followed by inhibition of the enzyme at higher drug concentrations. Under these conditions, ATPase becomes Mg^{2+}-dependent, which is indirect evidence that the heads break away from the inner membranes of the mitochondria (*103–105, 107, 109*).

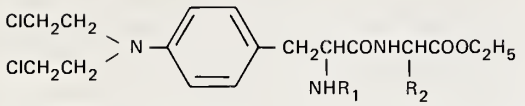
These data are confirmed by examination with an electron microscope, which shows that damage to the outer and inner membranes of the mitochondria is one of the earliest manifestations of the cytotoxic effect of sarcolysin on tumor cells. Damage in the ultrastructure of the endoplasmic reticulum and the nucleus occurs later (*96, 97*).

Thus two kinds of sarcolysin cytotoxicity were established by the works of Soviet scientists: *nuclear,* manifested by disrupted structures and functions of DNA and nuclear proteins; and *membrane,* caused by disturbances in the phosphorylating function of the inner membranes of the mitochondria, and by damage to the endoplasmic reticulum membrane and the transport processes through the plasma membrane. We believe the increase in selectivity of the antitumor effect of sarcolysin over that of its precursors is caused by the latter type.

In the early 1960's in the Soviet Union, many of the so-called "complex alkylating metabolites," dipeptides of sarcolysin with ethyl esters of various amino acids, amides and complex esters of phenylalkannic acids, were synthesized (*42, 56, 71–74, 139, 140*). (*See* text-fig. 2.)

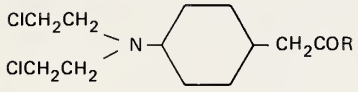
It was assumed that the terminal part of the carrier would selectively direct the alkylating "warhead" to the cells of one

Sarcolysin dipeptides



R_1	R_2	Preparation name
$-COCH_3$	$-CH_2C_6H_5$	Asaphan
$-COCH_3$	$-CH_2CH_2SCH_3$	Asameth
$-H$	$-CH_2CH_2SCH_3$	Sameth
$-COCH_3$	$-CH(CH_3)_2$	Asalin
$-H$	$-CH(CH_3)_2$	Salin
$-COCH_3$	$-CH_2CH(CH_3)_2$	Asaley

Amides and complex esters of *p*-(di-2-chloroethyl) aminophenylacetic acid (chlorphenacyl)



R	Preparation name
$-HCHC_6H_5COOC_2H_5$	Phenaphan
$-HCOOC_2H_5CH_2CH_2SCH_3$	Phenameth
– cholesterol	Phenesterin
– synestrol	Phenestrol

TEXT-FIGURE 2.—Complex alkylating metabolites.

tumor or another, depending on the characteristics of its structure and metabolism (*71, 72*).

Sarcolysin dipeptides with free amino groups are highly soluble in water and differ little from sarcolysin in toxicity and in spectrum of antitumor effect (*56*). The dipeptides with substituted amino groups and chlorphenacyl esters and amides are insoluble in solutions with low toxicity and a broad spectrum of antitumor action specific for each of them (*19, 36, 42, 65, 66, 72, 73, 124, 128, 129*). They differ from sarcolysin in pharmacodynamics, i.e., by tight and prolonged binding with components of normal tissues and tumors (*77, 78, 80, 88*). Although ^{14}C-astyron and ^{14}C-asaphan administered in adequate doses quickly enter normal tissues and tumors where they are bound tightly, they are slowly eliminated from the body. About half the peptides are located in the tissues and tumors in the free state in 4 days, but some are catabolized by peptidases into sarcolysin and the corresponding amino acid.

A specific tropism of sarcolysin dipeptides for the endocrine organs depends on the nature of the terminal amino acid. Thus the phenylalanine dipeptide of sarcolysin (asaphan) accumulates predominantly in the ovaries and that of tyrosine (astyron) in the thyroid gland (*77*). The capacity of the dipeptides for degradation by peptidases of tumor cells has been observed in vitro. About 10% of the peptide administered was decomposed into sarcolysin and amino acid after 3 hours of incubation with a tumor-cell suspension (*108*). Because the peptides are tightly bound to tumors and normal tissues, their toxic side effects (though not as severe) are more prolonged than those of sarcolysin. After administration of a single maximum tolerable dose (MTD) of sarcolysin, normal hematopoiesis begins in 3 days; however, after administration of an adequate dose of asalin, the first symptoms of fewer blood cells being produced appear in 2–3 days; normal production is not completely restored, even after 10 days. The antitumor effect of the sarcolysin dipeptides depends on the stereoconfiguration of each of the carriers; natural configuration is not essential for achievement of maximum activity (*139*).

Asalin, the valine dipeptide of sarcolysin, is now being used in the clinical setting (*92*). Other dipeptides and chlorphenacyl amides are still in the clinical study stage.

A high degree of tropism for tumor- and normal-cell membranes by sarcolysin dipeptides was observed by Romanova et al. (*105*). The dipeptides studied (salin, asalin, asaphan) are more potent inhibitors of respiration and phosphorylation than is sarcolysin; they react much like oligomycin rather than as uncouplers (*13, 103–105*). The supplementary aromatic ring or bulky aliphatic tail (side chain) of the terminal amino acid apparently facilitates the development of sarcolysin dipeptides in the hydrophobic regions of the inner membranes of the mitochondria. Then gradually moving to the interface with the water phase, they alkylate the nucleophilic centers of the enzymes or the electron and energy carriers (*9*).

The effect of the sarcolysin dipeptides on the structure and function of mitochondria, like the antitumor activity, depends on the stereoconfiguration of the carrier. Thus D,D-asaphan, therapeutically ineffective, cannot suppress respiration and phosphorylation of tumor-cell mitochondria, unlike the active antitumor compound L,L-asaphan (*110*).

Evidently, interference with the structure and function of mitochondrial membranes plays a significant part in the antitumor effects of sarcolysin dipeptides. On the other hand, a comparative study of the effect of embichin (HN2) and sarcolysin and its dipeptides on the structure of DNA and nuclear proteins has shown that the sarcolysin dipeptides clearly yield to the simpler chloroethylamines in capacity for alkylating DNA and forming cross-links between DNA and the nuclear proteins (*126*). The mutagenic effects of sarcolysin are stronger than those of its dipeptides (*66*).

Therefore, the membrane component of these antitumor compounds has the highest total cytotoxic effect. A number of researchers (*39, 51, 57, 59, 61, 120–122, 145, 146*) have found a similar dependence of certain chlorphenacyl derivatives and sulfonic acid esters between the structure of the cytotoxic group carrier and the nature of the effect on mitochondrial enzymes.

These works sustained the hypothesis that the inhibiting effect of the alkylating compounds on the electron transport system and oxidative phosphorylation is determined by two parameters: the dissociation constants and the distribution coefficient between the water and the lipid solvents. A decisive role in the reaction with specific enzymes of the mitochondrial membrane is played thus by the ratio between the polar and hydrophobic portions of the alkylating compound molecule. (*See* text-fig. 3.)

Compounds with open carboxyl and amino groups are found in the polar portion of the membrane or on the interface of the polar and hydrophobic regions interacting with the nucleophilic groups of the enzymes. Compounds with masked polar groups are submerged in the hydrophobic portions of the membrane; they alkylate other enzymes, as shown by inhibitor analysis (*146*).

Among the complex alkylating metabolites, the hormonocytostatics (in which the chlorphenacyl molecule is combined by a complex ester bond with natural or synthetic sex hormones) are of interest. Hormonocytostatics are active antitumor compounds with properties similar to hormones and alkylating agents (*125*). Their effect, directed mainly toward hormone-dependent tumors, depends on the sex of the animal host.

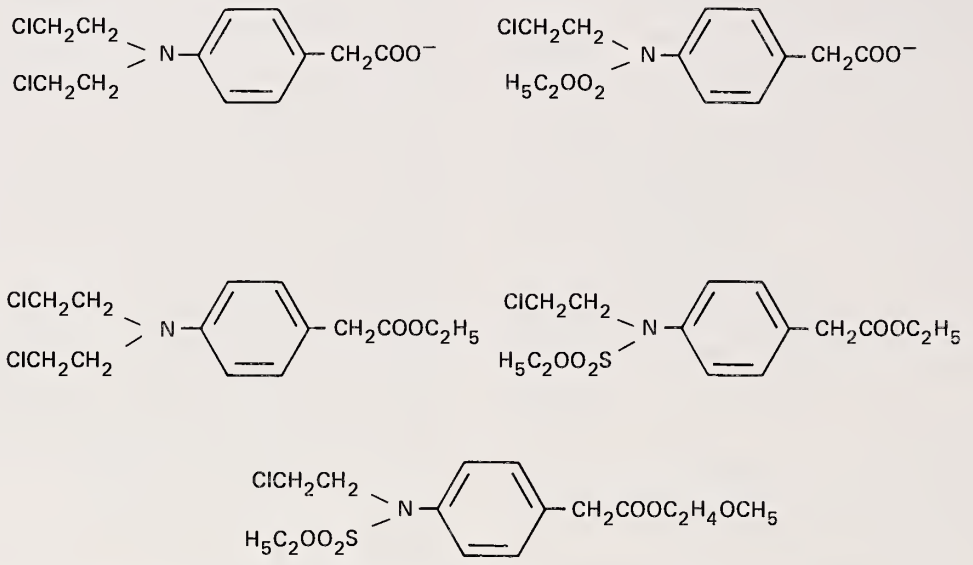
Evidently, these compounds should be considered as transport forms of chlorphenacyl and the corresponding hormone. They are gradually decomposed in the body and have direct and endocrine system-mediated effects on a tumor.

Ethyleneimines

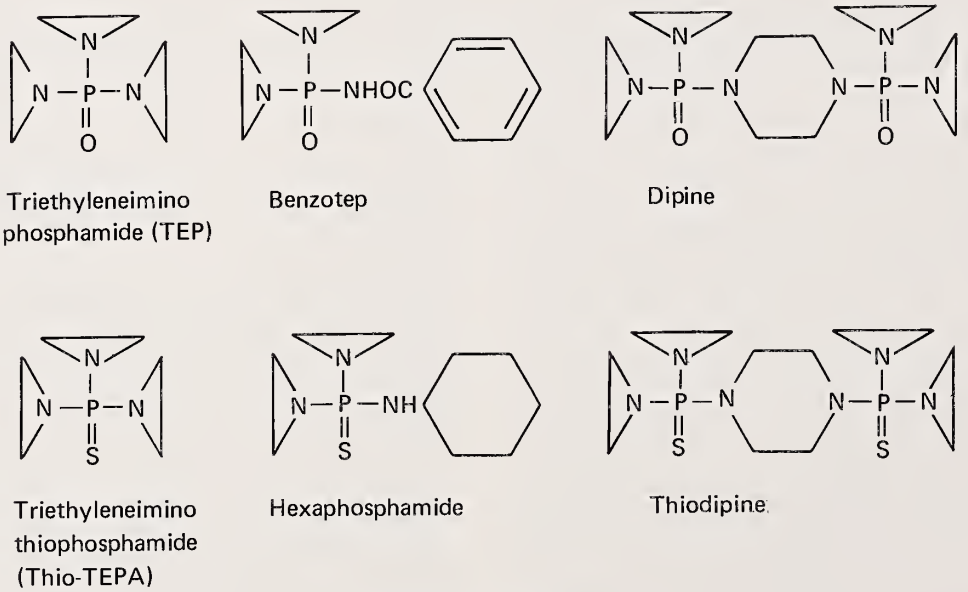
Ethyleneimine derivatives of phosphoric and thiophosphoric acids (TEPA and thio-TEPA) are used in the Soviet Union for treatment of malignant diseases of the blood and tumors in various sites (text-fig. 4). Pyrimidine, piperazine, benzoic acid, purine, and cyclohexane residues are used as carriers of cytotoxic groups (*62, 95, 111, 115, 136, 137*).

The selectivity and spectrum of their antitumor effect depends on the structure of the carrier and the nature of the substituents in the rings (*60, 81, 95, 111, 136*). As with haloid alkylamines, selectivity of the antitumor effect of this class of compounds apparently is determined by the ratio between the polar and hydrophobic portions of the molecules.

The mutagenic effects of the ethyleneimines, discovered before they began to be used as antitumor compounds (*99, 100*), are caused by their capacity for alkylating the DNA molecule (*47, 48, 65*). The process of DNA alkylation proceeds as a second-order reaction, in which the denatured portions of the double helix DNA molecule act as catalysts in opening the ethyleneimine ring. There are indications that some ethyleneimines are capable of interfering with tumor-cell energetics (*51*).



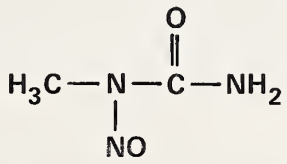
TEXT-FIGURE 3.—Alkylating compounds: *Upper portion,* charged; *lower portion,* uncharged.



TEXT-FIGURE 4.—Ethyleneimines.

Nitrosourea Derivatives

During the last decade, the attention of chemotherapists specializing in oncology has been directed toward certain nitrosourea derivatives. The antitumor activity of the *N*-alkyl derivatives of nitrosourea, the well-known "supermutagens" (*101*), was discovered in the Soviet Union at the beginning of the 1960's by N. M. Emanuel and colleagues (*142–144*). *N*-Methylnitrosourea (MNU), which received a positive evaluation in clinical research, proved effective in patients with undifferentiated cancer of the lungs and generalized lymphogranulomatosis (*92*).



N-methyl-N-nitrosourea

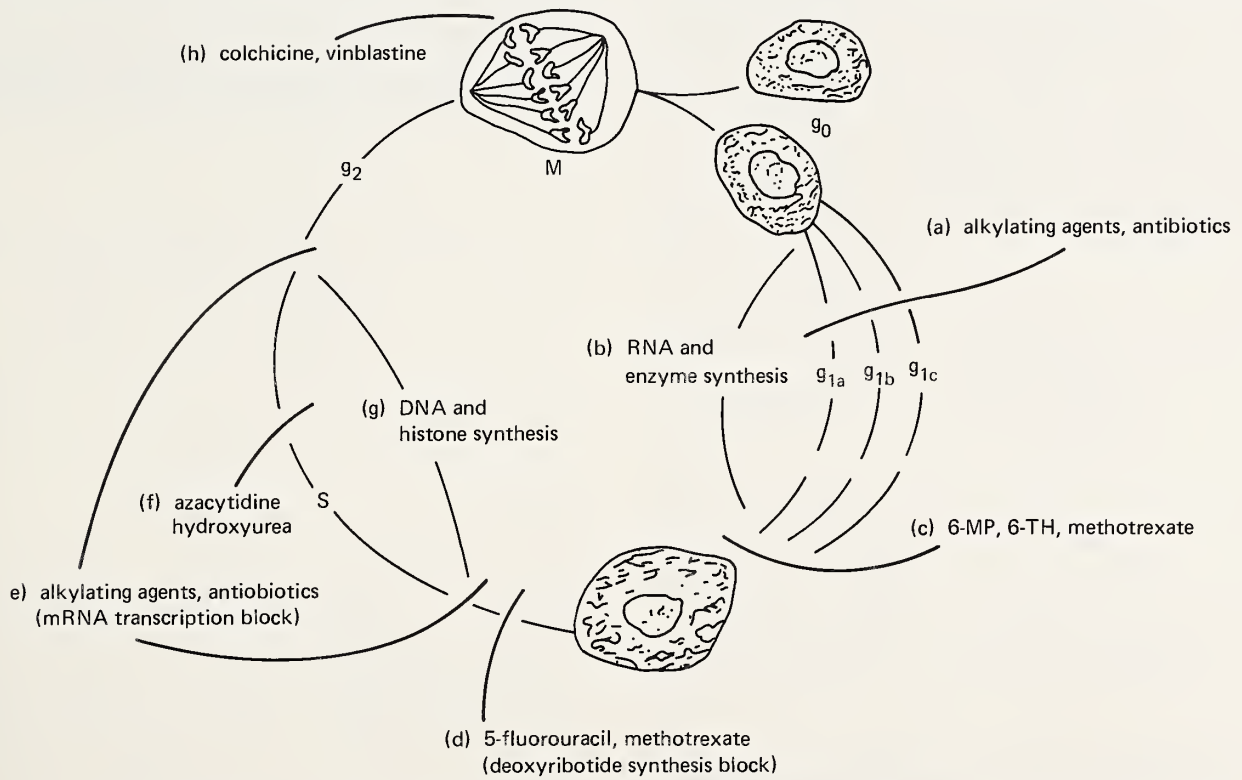
The molecular mechanisms of action of nitrosourea derivatives have not been studied sufficiently. It is known only that, after a number of metabolic conversions in the cell, they become methylating agents. The guanine and cytosine residues of DNA and of various RNA fractions are subjected to methylation. Carbamoylation of DNA molecules by the action of *N*-alkylnitrosourea has also been detected. Besides methylation and carbamoylation of the DNA molecules, leading to mistakes in their replication and mutagenic effects (*32–36, 101, 117–119*), a strong inhibition of the transcription process by alkylnitrosoureas was found. Not only damage of the template activity of DNA but the reaction of the compounds with RNA polymerase (leading to its inactivation) are the basis for this effect (*86*). Moreover, the interference of alkylnitrosoureas with the translation has also been demonstrated, the cause of which is apparently related to alkylation of mRNA and carbamoylation of the amino groups of some ribosomal proteins by the isocyanates (*32*).

Effects of Alkylating Agents on the Cell Cycle

The works of Soviet scientists suggest many alkylating agents to be called "cycle-dependent" antitumor compounds (*29, 31, 43, 79, 112, 123*).

Cells in the "critical periods" of the cell cycle, namely, in the first half of the G_1 phase and the beginning and end of the S and G_2 phases (text-fig. 5), are the most sensitive to the effects of alkylating agents. As is well known (*10*), reprogramming of the cell genome, synthesis of new mRNA and proteins necessary for transition to the succeeding phase of the cycle, takes place precisely during the critical periods of the cycle.

Evidently, alkylating agents entering a tumor cell at these times can effectively block the genome reprogramming process because of damage not only to the DNA matrix, on which new mRNA is synthesized, but also to components of the protein-synthesizing system. The results are mitotic blocks and synchronization of the cell population (*8, 10*). A mitotic block in the transition of the cells from the S phase to G_2 and from G_2 to mitosis, induced by alkylating agents, is expressed by the



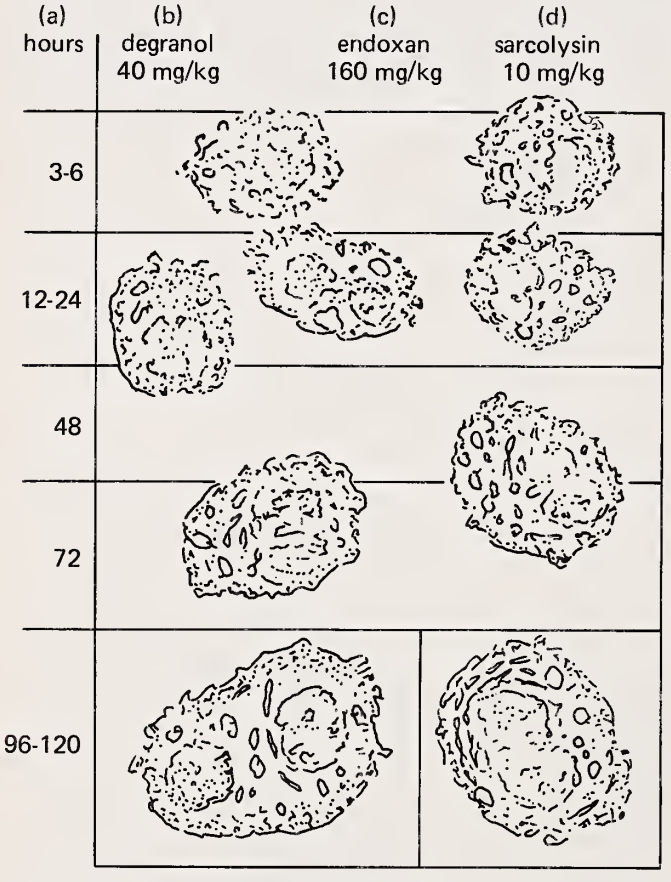
TEXT-FIGURE 5.—Diagram of cell cycle (from Mueller) and its critical periods of increased sensitivity to the action of antitumor compounds.

appearance of giant cells, which increase with time and which make up a considerable portion of the cell population with massive doses of the compound (*6, 30, 68, 92*). The fate of such giant cells depends on the duration of the block (*30*) (text-fig. 6).

Thus molecular mechanisms of cytotoxic action of antitumor alkylating compounds are made up of many components:

1) Damage to DNA structure and template functions in the replication and transcription processes, the results of which are mutations and the unbalanced growth syndrome (mitotic block);
2) Interference with the translation process, as a consequence of alkylation of the mRNA, rRNA, tRNA, and ribosomal protein molecules;
3) The ability of concentrating in the membrane structures of the cells and of disrupting their functions, electron and energy transport, and active transport of cations, amino acids, and other substances.

Individual aspects of the cytotoxic effects enumerated are expressed differently in different compounds, depending on the nature of the cytotoxic groups and the structures of their carriers. This provides a basis for synthesis of compounds, with a predominance of one component of the cytotoxic effect or another, specifically directed toward treatment of specific types of tumors. Intensive work in this direction is being done in the Soviet Union.



TEXT-FIGURE 6.—Unbalanced growth syndrome (appearance of giant cells) caused by administration of single MTD of alkylating antitumor compounds (*68*).

Research under way in the Siberian Section of the U.S.S.R. Academy of Sciences includes the synthesis of alkylating derivatives of tRNA (text-fig. 7) and of synthetic oligoribonucleotides, fragments, or mRNA (*3–5, 23*).

It has been shown that *N*-chlorambucil-phenylalanyl-tRNA (text-fig. 7, upper) specifically and covalently binds to peptidyltransferase sites of a 50S ribosomal subunit, whereas the oligo(A) or oligo(U), carrying alkylating groups, binds in place of mRNA with the 30S subunit (text-fig. 7, lower). The covalently bound polyribonucleotides and oligoribonucleotides form a ternary mRNA–ribosome–tRNA complex capable of synthesizing polypeptides (*22*).

These types of alkylating polynucleotides and oligonucleotides have not been tested as possible antitumor compounds. However, the idea of controlled and specific alkylation of specific sites of biologically important polymers can be useful in the search for antitumor compounds of the future.

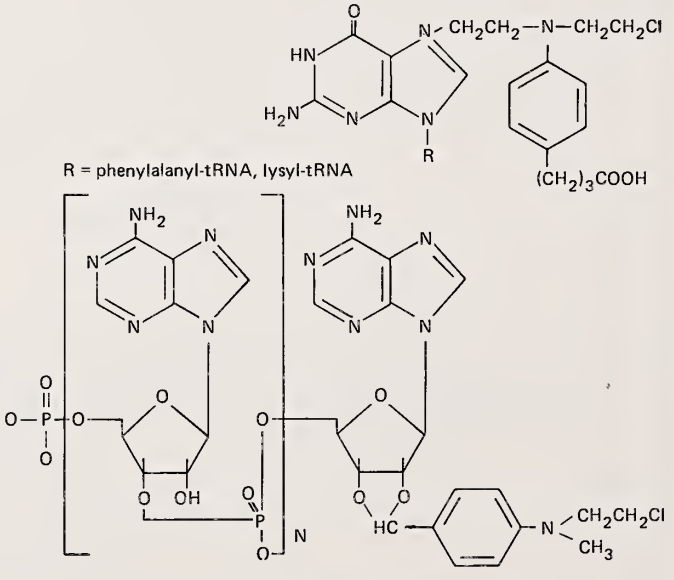
ANTIMETABOLITES

Folic Acid Analogues

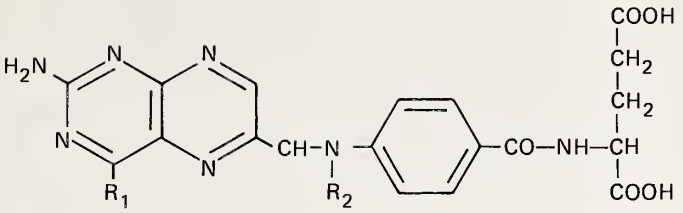
Among the antitumor antimetabolites, the first used clinically were the folic acid analogues aminopterin and methotrexate that are powerful dihydrofolate reductase (DHFA-reductase) inhibitors (text-fig. 8). The high toxicity of these preparations forces investigators to search for new folic acid analogues, with higher selectivity of antitumor activity (*112*).

In searching for such compounds in the early 1950's, a series of aminopterin derivatives was synthesized in which glutamic acid was replaced by aminoadipic, aminopimelic, aminosuberic, and aminoazeleic acids (*54*). These folic acid analogues proved to be much less toxic than aminopterin, and one of them (adipinaminopterin) is highly effective against the murine leukemias (*52, 53*).

Our finding that the selective inhibition of incorporation of ^{14}C-formate in tumor cell nucleic acids that led us to believe the compound was a DHFA-reductase inhibitor (*55*) was con-



TEXT-FIGURE 7.—Alkylating derivatives of tRNA.



R_1	R_2	
OH	H	Folic acid
NH_2	H	Aminopterin
NH_2	CH_3	Methotrexate

TEXT-FIGURE 8.—Folic acid analogues.

firmed later (*131*). Adipinaminopterin inhibits DHFA-reductase in spleens of mice that had been infected with Rauscher leukemia virus, and it delays development of the leukosis. Unfortunately, the preparation has not been studied clinically.

Subsequently, research for these analogues and their mechanisms of action was begun in a number of laboratories (*117*). Thus V. M. Berczovski and colleagues (*15, 16*) attempted to disrupt the structure of those functional groups that catalyze the pyrazine ring and *N*-5 and preserve the pyrimidine ring necessary for binding to the apoenzyme.

For this purpose, a series of pyrimidine analogues of folic acid, with various substituents in the 2, 4, and 6 positions and analogues without glutamic acid, was synthesized. All the analogues are strong DHFA-reductase inhibitors. For some, the inhibition constants (K_i) for partially purified DHFA-reductase from rat livers were determined (table 1).

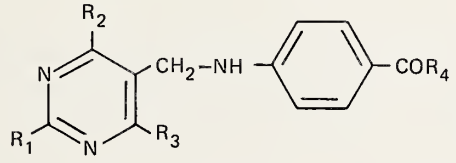
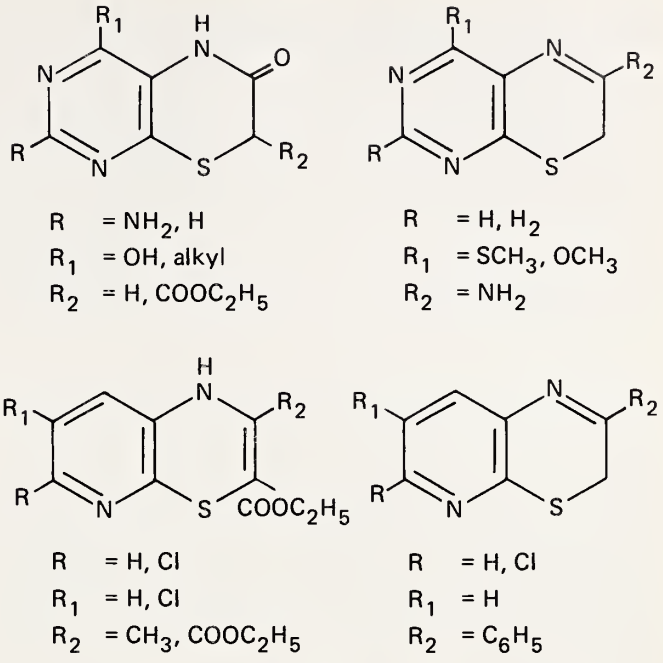


TABLE 1.—*Inhibition of DHFA-reductase by folic acid analogues*

	Analogue			K_i (5×10^{-6} M)
I	$R_1 = R_2 = OH$	$R_3 = CH_3$	R_4 = glutamate	1.70
II	$R_1 = R_2 = OH$	$R_3 = CH_3$	$R_4 = OH$	1.60
III	$R_1 = R_2 = OH$	$R_3 = H$	$R_4 = OH$	1.00
IV	$R_1 = R_2 = OH$	$R_3 = H$	R_4 = glutamate	4.90
V	$R_1 = CH_3$; $R_2 = OH$	$R_3 = H$	R_4 = glutamate	5.60
VI	$R_1 = R_2 = NH_2$	$R_3 = H$	R_4 = glutamate	0.32
VII	$R_1 = CH_3$; $R_2 = NH_2$	$R_3 = H$	R_4 = glutamate	—

Contrary to expectations, the 2,4-dioxypyrimidine analogue IV inhibits DHFA-reductase more strongly than the 2,4-aminopyridimide analogue VI. The electron donor methyl group in position 2 weakens the inhibiting properties of analogues V and VII. The methyl group in position 6, on the other hand, reinforces the binding strength of analogues I and II to the enzyme. Some pyrimidine analogues of folic acid possess antitumor activity.



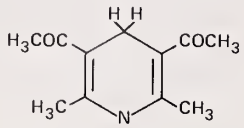
TEXT-FIGURE 9.—Pyrimidothiazines:DFHA-reductase inhibitors.

Research on folic acid analogues is proceeding successfully with the derivatives of pyrimidothiazines (*113, 114*), pyrazinothiazines (*87*), and pyridothiazines (*76*). The inhibitory properties of the pyrimidothiazines with respect to DHFA-reductase are reinforced by insertion of OH, OCH_3 or NH_2 in the C-4 and C-6 reactive groups (text-fig. 9). Opening of the thiazine ring leads to complete loss of activity. The pyridothiazine, 2-chloro-6,7-dicarbethoxy-5-*N*-pyridothiazine, which inhibits growth of a number of transplanted tumors in mice and rats, is a potent DHFA-reductase inhibitor; 3-chloro-substituted pyridothiazine analogues inhibit neither DHFA-reductase nor growth of animal tumors (*67*). A series of analogues of pyrimidothiazines and pyridothiazines were selected as inhibitors of DHFA-reductase and aminopterin reductase (an enzyme-reducing aminopterin), making them harmless (*1, 2*). These analogues effectively inhibit the growth of tumors resistant to aminopterin, some of which are now being studied (*112*).

In the Ural Polytechnical Institute, research on DHFA-reductase inhibitors is being performed with derivatives of *N*-4-(2-naphthyl)-methyl-*p*-aminobenzoylglutamic acid (*40, 98*).

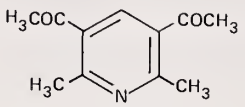
Some analogues of nicotinamide and dihydronicotinamide, synthesized at the Institute of Organic Synthesis, Latvian S.S.R. Academy of Sciences, and with known antitumor activity (*45*), revealed a similarity to the antifolic compounds in

their ability to inhibit DHFA-reductase (*11*).



(I)

2,6-dimethyl-3,5-diacetyl-1,4-dihydropyridine



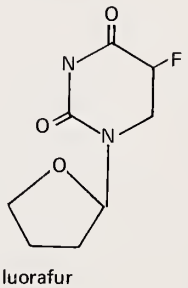
(II)

2,6-dimethyl-3,5-diacetyl-pyridine

Both nicotinamide analogues suppress biosynthetic reactions accompanied by transport of single-carbon fragments and synthesis of purines, thymidylate, and serine in tumor cells (*12, 46*). The most likely site of the metabolic block was DHFA-reductase. To test this proposal, enzymatic synthesis of two NADP analogues, 2,6-dimethyl-3,5-diacetyl-ADP and thio-NADP was done. Results of kinetic experiments with DHFA-reductase demonstrated that they are rather strong competitive inhibitors of the enzyme (*11*). It is clear that in tumor cells, nicotinamide analogues are converted into NADP analogues, which are true antimetabolites and DHFA-reductase inhibitors.

Pyrimidine Nucleoside Analogues

Work on the synthesis of new antitumor antimetabolites, the analogues of pyrimidine and purine nucleosides, has expanded considerably in the Soviet Union in recent years (*26, 40, 138*). The deoxyuridine analogue, fluorafur [2] (ftorafur; *N*-1-furanidyl-5-fluorouracil), synthesized by Hiller and colleagues (*26*), is effective in the treatment of tumors of the gastrointestinal tract (*18, 92, 151*). Fluorafur is structurally similar to fluorodeoxyuridine, but it contains a tetrahydrofuran ring in place of the deoxyribose. Since there is no hydroxyl group in this ring, fluorafur cannot be converted into a nucleotide without preliminary cleavage of the nucleoside bond.



Fluorafur

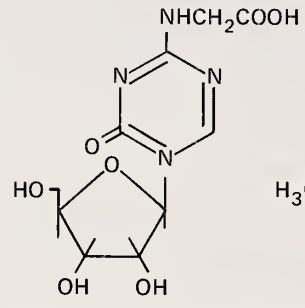
Therefore, having begun study on the mechanism of its action, D. V. Meiren and I (*82, 83*) proceeded on two assumptions: 1) Either fluorafur is active per se in the nucleoside form, or 2) the nucleoside bond in it is broken, with release of 5-fluorouracil, which undergoes lethal synthesis in the cell.

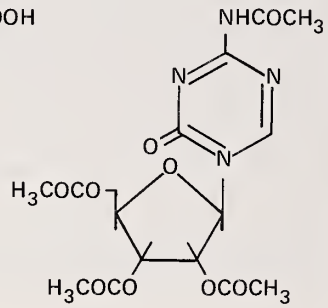
[2] Since this monograph went to press, the preferred term for this compound was changed to ftorafur. The terms are used interchangeably in this publication.

In experiments in vitro, fluorafur, even in high concentrations (1 mmole), hardly suppresses de novo thymidylate synthesis in ascites tumor cells and is not decomposed by the action of phosphorylase and hydrolase. However, in dilutions, by acidification of the medium or by heating, the nucleoside bond in the fluorafur molecule is broken, and free 5-fluorouracil is found in the solution. Simultaneously, with accumulation of 5-fluorouracil in the mixture, the bacteriostatic activity increases. All this permits us to consider fluorafur as a transport form of 5-fluorouracil, which agrees well with its pharmacologic properties and spectrum of antitumor effect (*83, 92, 130*).

More detailed study on the metabolism and activity of fluorafur by Japanese scientists confirmed data on its conversion to 5-fluorouracil. However, this conversion is not accomplished chemically, as was assumed initially, but enzymatically by oxidative enzymes of the endoplasmic reticulum.

Among the pyrimidine nucleoside analogues with antitumor activity, there is a definite interest in the derivatives of 6-azacytidine, glyacytidine, and tetracytidine, synthesized by V. P. Chernetski and colleagues (*50, 138*). The functions of 6-azapyrimidine ribosides have been studied well (*150*). They are converted into nucleotides, which are potent inhibitors of orotidylate decarboxylase, by the cellular uridine kinase.

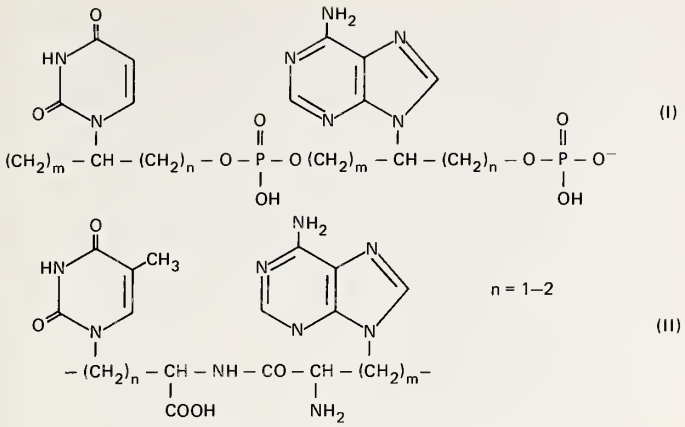




In the study of the effect of glyacytidine (above left) and tetracytidine (above right) on de novo synthesis of nucleic acid pyrimidines in ascites tumor cells, it was shown that these antimetabolites, unlike 5-fluorouracil, inhibit the incorporation of labeled pyrimidine nucleotides in RNA but not in DNA. The nature of the C′4 substituent of the azapyrimidine ring has a significant effect on the inhibiting properties of the analogues (*9*). 6-Azacytidine is a weak inhibitor of ^{14}C-orotic acid incorporation in tumor cell RNA ($K_i = 5 \times 10^{-4}$ M). Acetylation of ribose and amino group base hydroxyls in the tetracytidine molecule does not change its ability to suppress orotidylate decarboxylase, in comparison with 6-azacytidine. Substitution of oxygen in C′4 of the azapyrimidine ring by the glycine residue in the glyacytidine molecule sharply increases its inhibitory properties ($K_i = 5 \times 10^{-6}$ M). The azapyrimidine nucleotide C′4 apparently participates in binding the analogues to the active center of orotidylate decarboxylase.

Oligonucleotide Analogues

S. A. Hiller and colleagues (*25, 27*) synthesized analogues of the oligonucleotides for use as potential antitumor com-



TEXT-FIGURE 10.—Model oligonucleotides.

TABLE 2.—*Relationship between structure of pyridoxal phosphate (PP) analogues and their inhibitory properties*

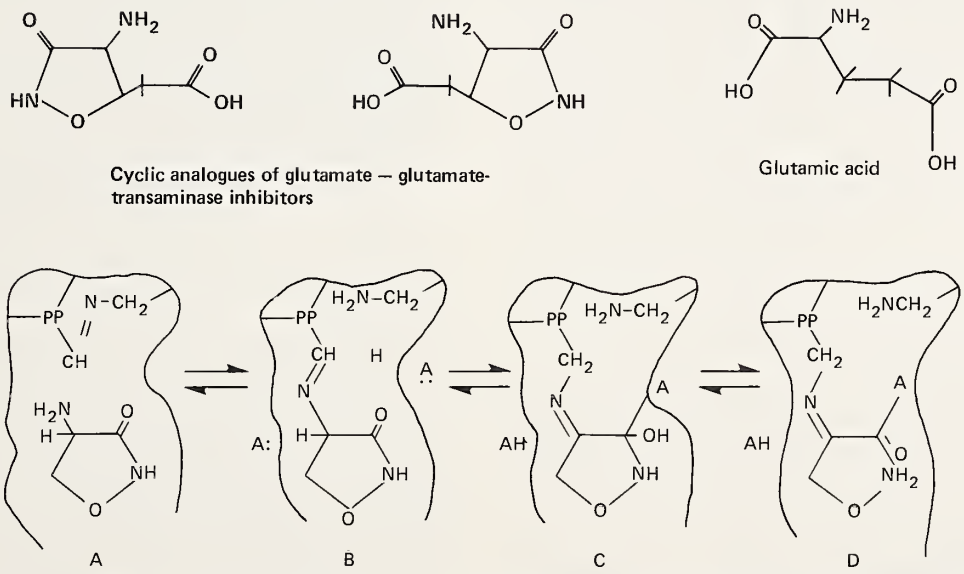
Coenzyme analogue	Inhibition, %			Inhibition constant, K_i (M)	
	SSH	CTS	GDC	SSH	GDC
3-Deoxy-PP	33	20	35	4.5×10^{-4}	1.0×10^{-4}
2′-Isopropyl-PP	65	50	—	1.1×10^{-4}	—
2′-Phenyl-PP	71	70	70	0.8×10^{-4}	1.7×10^{-5}
6-Methyl-PP	60	44	65	1.2×10^{-4}	6.9×10^{-5}
5-*N*-5-carboxyethyl-PP	27	22	30	1.2×10^{-3}	3.3×10^{-4}

pounds. Their synthesis is based on the theory of interference with transfer of genetic information, by means of blocking or activation of specific sites of the tumor cell genome by complementary oligonucleotides.

One group of oligonucleotide analogues consists of chains containing N_1 (N_9)-1,4-dioxybutyryl-2-pyrimidines and N_1 (N_9)-2,5-dioxypentyl-2-pyrimidines and purines. [*See* text-fig. 10(I).] Another group includes oligonucleotides, consisting of pyrimidyl-N_1 and purinyl-N_9-amino acids joined by peptide bonds [text-fig. 10(II)]. The model oligonucleotides are capable of hybridization with nucleic acids.

Considering that other amino acids similar to L-alanine should assume a bent conformation in the active center of the enzyme, the authors synthesized cyclic analogues of glutamic acid. Some of them proved to be strong and specific inhibitors of glutamate-aspartate transaminase. Another group of conformational inhibitors of pyridoxal enzymes, the esters of carbohydroxamic acids, are like "split" cyclic amino acid analogues. They assume the required conformation in reaction with the active center of the enzyme and are irreversibly bound to it (*116*).

Finally, some analogues of pyridoxal phosphate that are competitive inhibitors of the pyridoxal enzymes should be mentioned: glutamate decarboxylases, serine sulfhydrylases, and cystathionine-β-synthetases. These compounds compete with pyridoxal phosphate for the binding sites in the active center of the apoenzyme (*44, 84*) (table 2). Of the pyridoxal enzyme inhibitors, only cycloserine was investigated as an antitumor compound (text-fig. 11). It displayed a definite activity against animal leukemias (*24*) and is now undergoing clinical study. The other analogues are awaiting study as possible antitumor compounds.



TEXT-FIGURE 11.—Diagram of mechanism of inhibition of L-alanyl transaminase by L-cycloserine (*116*); PP=pyridoxal phosphate.

Thus study on the mechanisms of action of the analogues of folic acid, nicotinamide, and pyrimidine nucleosides and the relationship between their structure and inhibiting properties has led to the synthesis of a number of new, effective antitumor antimetabolites in the Soviet Union. Efforts at interference with the regulation of gene activity of tumor cells by model oligonucleotides also are promising. Finally, an efficient search for specific and irreversible inhibitors of the pyridoxal enzymes with data based on the structure of their active centers should also lead to synthesis of effective antitumor compounds. These enzymes are important in the key biosynthetic processes and amino acid metabolism of tumor cells.

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Trends in Searching for Antitumor Compounds

M. N. Preobrazhenskaya[1]

At present, two approaches can be used in the search for antitumor compounds. Various drugs of synthetic and natural origins are being studied, synthesized, or isolated for some other purpose and come within the sphere of attention of investigators looking for antitumor compounds. Controlled synthesis and comprehensive study are being done on substances which, for one reason or another, may have antitumor activity. Most of the substances being studied in the U.S.S.R. for antitumor properties come from laboratories working on controlled syntheses of potential antineoplastic compounds.

In planning such work, one proceeds from conceptions of the possible mechanisms of action of antitumor compounds and their individual structures, which make possible the activity of the proposed biochemical processes. Expansion of our knowledge on the mechanisms of action of compounds already discovered is of great importance. The purpose of many investigations is to obtain a more active and less toxic analogue of an existing drug.

Efforts toward synthesizing antitumor agents efficiently are now based primarily on existing data on the mechanisms of direct action of a compound on the cell and, to a considerably lesser extent, on conceptions of the causes of medicinal selectivity of cytotoxic drugs. It is difficult to find an approach to a specific search for homeostasis regulators. The action of most antitumor compounds is involved with their suppression of various stages of nucleic acid metabolism. Several types of action on DNA and RNA metabolism are known; a compound can react directly with nucleic acids by disrupting their normal functioning ability and indirectly by acting on enzymes responsible for their biosynthesis and functions. Alkylating compounds react directly with the nucleic bases of the DNA double helix. Some antitumor antibiotics block the DNA matrix by covalent binding with the DNA and hamper the action of polymerases. Intercalation of the compound is possible between the nucleic bases in the DNA or RNA chain, which also prevents normal functioning of the enzymes.

The activity of nucleic acids is disrupted if a natural component in the DNA or RNA chains is replaced by a modified one. In some instances, replacement of the cofactor functions of normal nucleotides in nucleotide coenzymes by their modified analogues takes place (5-fluorouridine diphosphate, sugars, tubercidin analogue of NAD).

The action of many antitumor antimetabolites is based on 1) their reaction with proteins, 2) enzymes accomplishing biosynthesis of DNA or RNA components, 3) their catabolic conversions, and 4) the synthesis of biopolymers. The search for this kind of compound among the analogues of the nucleic bases, nucleosides, deoxynucleosides, and coenzymes is promising. An inhibitor may react with the active center of an enzyme (the types of reaction can be diverse), or it may have allosteric or repressor effects on the enzyme.

For a proposed antitumor agent to react with enzymes, a structural analogy is necessary in which the preparation usually remains as a substrate for some enzymes (for example, lethal synthesis enzymes) and becomes an inhibitor for others. With an idea of the structure of the active center of an enzyme that is especially important to suppress, a more direct approach is possible. Thus successful development of antitumor compounds through chemistry is based on fundamental molecular and biologic concepts.

The action of another group of drugs concerns their suppression of mitoses. Reaction with the protein of the spindles and microtubules is important here, although the antitumor effect apparently also depends on suppression of certain other biochemical processes.

An intensive search has been made for substances capable of selective degradation of those structures particularly necessary to the tumor cell. We should also mention here work involving enzymes that degrade amino acids (e.g., asparaginase) and the efforts to discover hexokinase inhibitors. The increased activity of the latter in a tumor cell determines whether small amounts of glucose can be used by tumors.

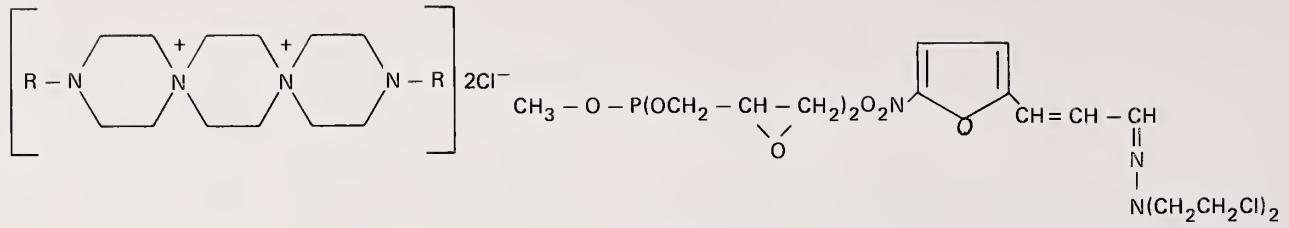
Also in recent years, numerous investigations have dealt with the effects of cell membranes on malignant degeneration of cells, efforts to find a way to regulate malignant growth, and with substances that react with membranes or their components. Work in this direction is closely involved with basic research on membrane structure and function.

The search for antitumor compounds that indirectly affect tumor growth, homeostasis regulators, and immunity stimulators is especially interesting because it includes investigating biopolymers of natural origin. The study of regulatory factors and the creation of analogues and antagonists of natural regulators such as steroids, polypeptide hormones, cyclonucleotides, and others, is important.

ALKYLATING COMPOUNDS

The study of chemical and biologic properties of compounds containing an alkylating fragment (most often, a *bis*-2-chloroethylamino group or ethyleneimine group) is the usual direction Soviet investigators proceed in their initial research in new product development. Synthesis and the investigation of substances, in which the alkylating fragment is combined with various organic molecules, is being done at the S. Ordzhonikidze All-Union Scientific Research Chemical-Pharmaceutical Institute, U.S.S.R. MMP [Ministry of the Medicinal Industry; (VNIKhFI)], Institute of Organic Synthesis, Latvian S.S.R. Academy of Sciences (IOS), Kiev Scientific Research Institute of Pharmacology and Toxicology, Ukrainian S.S.R. Ministry of Public Health (KNIFT), S. M. Kirov Ural Polytechnic Institute (UPI), Leningrad Technological Institute

[1] Head, Laboratory of Chemical Synthesis, Cancer Research Center, Moscow, U.S.S.R.



$R=CH_2CH_2Cl$ Spirazidine
$R=CH_2CHClCH_2OH$ Prospidine

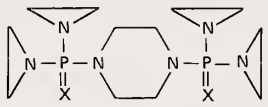
Cidiphos

Nifuron

(LTI), Institute of Biochemistry, Lithuanian S.S.R. Academy of Sciences, Institute of Elemental Organic Compounds, U.S.S.R. Academy of Sciences (INEOS), and other institutes.

Among the new alkylating compounds, prospidine has a broad therapeutic effect and suppresses hematopoiesis slightly. In a number of prospidine analogues, the antitumor effect is at a maximum in the presence of three spiropiperizine cycles (*64, 66, 67, 81, 92*). Pharmacokinetic properties of ^{14}C-prospidine were investigated by Bogomolova et al. (*9*).

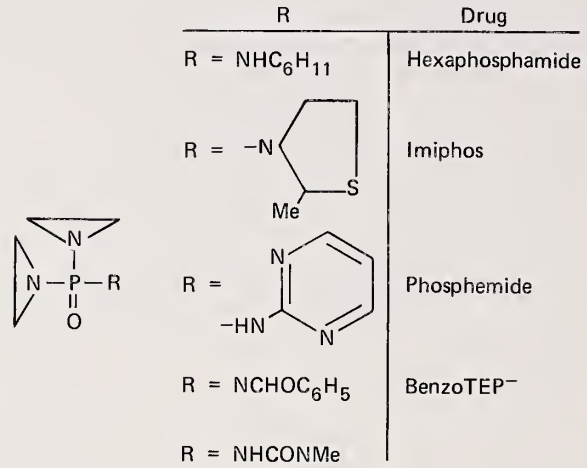
The compound nifuron is *bis*(2-chloroethyl)-hydrazone-5-nitro-2-furylacrolein (*24*). *Bis*(2-chloroethyl)-hydrazones and methylhydrazones of other heterocyclic aldehydes, as well as substitution hydrazides of various carboxylic acids, have been studied. An alkylating epoxy group is the active principle of the compound cidiphos, a diglycidyl ester of methylphosphoric acid (*89*).



X–O Dipine
X–S Thiodipine

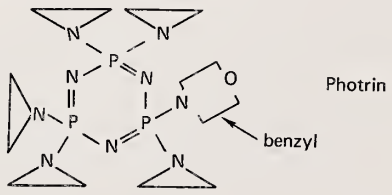
Data on the antitumor activity of the triethylenetriamide of phosphoric acid and of its thio-analogue (thio-TEPA) led to widespread synthetic studies of compounds of this type. In these structures, substitution of one or two ethylimine groups on a residue of an aromatic, fatty, or heterocyclic amine can lead to a change in the therapeutic index and specificity of action of the compound. Dipine and thiodipine have been introduced into medical practice. Hexaphosphamide, effective in the treatment of chronic myeloleukemia, has a therapeutic action in forms of the disease resistant to myelosan or busulfan (*43, 44, 90*). Diethyleneimides of phosphoric acid have been synthesized; the structure includes thiazolidine or thiomorpholine residues. The toxic effect may be mitigated by the protective action of the β-aminoethylmercaptan fragment of the substitute. There is the greatest interest in imiphos, which is used successfully in the treatment of erythema (*23*). *N*-Phosphorylated derivatives of urea were also investigated (*24*).

Of the heterylamidophosphoric ethyleneimides obtained, phosphemide has been approved for medical use (*65, 90, 92*); its derivatives, containing various substitutes in the pyrimidine ring, as well as analogous aminopyridine, aminopyrazine, and aminopyridazine derivatives, are active. After discovery of the high activity of the benzoylidiethylenetriamide of phosphoric



acid (benzo-TEPA), derivatives were obtained containing various substitutes in the benzoyl nucleus; the effect of the nature of the substituents and their positions on biologic activity were revealed. Compounds with meta-substituted benzo-TEPA and fluoro and iodo derivatives of benzo-TEPA are being studied (*81*).

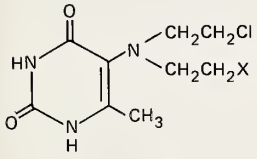
Research involving derivatives of cyclotriphosphazatriene (triphosphonitrile chloride), which can be called phosphoric analogues of melamine, is extremely interesting. The biologic effect depends on the number of ethyleneimine substituents and the nature of other substituents and their locations in the ring. Compounds containing (besides ethyleneimine groups) morpholine or pyrrolidine cycles are the most active, and those containing amino acid residues are less so. Photrin has been approved for use in leukemias and dermatorrhagias (*54, 68, 90*).



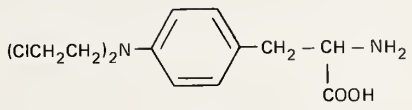
The possibilities of synthesis of potential antitumor compounds containing an alkylating group bound to the molecule of an organic compound are practically unlimited. The com-

plexity consists, not of obtaining more and more new representatives of this class of compound, but in the search for methods that can show the differences in the molecular bases of the biologic action of these preparations and the advantages of some types of derivatives over others.

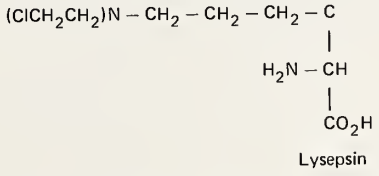
The role of the compound to which the alkylating fragment is bound for manifestation of antitumor effect is especially significant for alkylating derivatives of biologically important compounds, i.e., amino acids, nucleic bases, coenzymes, and others. In 1948, L. F. Larionov of the U.S.S.R. Academy of Medical Sciences proposed the idea of the possible antitumor activity of a metabolite having an alkylating group. Research on this suggestion was done at IECO, LTI, UPI, the Institute of Biochemistry, Lithuanian S.S.R. Academy of Sciences, INEOS, and at other institutions. The dopan or sarcolysin molecule was modified, with the chlorine atom being replaced by other halogens (e.g., ftorodopan); the alkylating group in sarcolysin was inserted into another position, and additional substituents were introduced (*28, 36, 88, 94, 100*).



Dopan X = Cl
Ftorodopan X = F



Sarcolysin



Lysepsin

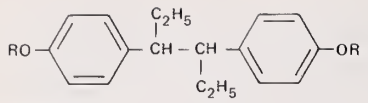
Several authors have reported on the alkylating derivatives of other amino acids, e.g., lysine, tyrosine, and glutamic acid (*28, 29, 31, 88, 93*). They expressed the hypothesis that derivatives of a natural catabolite containing an alkylating fragment could be considered as potential antimetabolites; the activity displayed was explained as both the ability of the compound to alkylate some component of the cell and to compete with the corresponding metabolites. Unfortunately, no method could be found that would permit separation of these effects and confirmation of this premise.

Numerous studies were made on complex structures, derivatives of alkylating compounds and amino acids or peptides, lipids, and steroid hormones, etc. (*28, 56, 58, 60, 76, 88*). For sarcolysin, derivatives with various protective groupings at the amino group were obtained, and the activity of the *N*-acetyl derivatives was demonstrated. Ethyl esters of *N*-acetylsarcolysylphenylalanine (asaphan), *N*-acetylsarcolysylleucine (asaley), *N*-acetylsarcolysylmethionine (asamet), *N*-acetylsarcolysylvaline (asalin), *N*-acetylsarcolysyltyrosine (astyron), *N*-acetylsarcolysyldioxyphenylalanine (asadophan), and other dipeptide analogues have been investigated. These compounds have less toxicity and apparently greater selectivity of action than does sarcolysin, with the structure of the unalkylated component frequently determining the manifestation of antitumor effect in this type of derivative (*45, 88, 94*). Thus the ethyl ester of 4-*bis*-(2-choroethyl)aminophenylacetyl-β-phenylalanine (phenophane) displays antitumor activity, whereas the corresponding derivative of β-phenyl-β-alanine does not (*98*). The optical configuration of the amino acid is of great importance. Using the example of sarcolysin again, Sof'ina et al. demonstrated that the chemotherapeutic activity of the L-isomer (melphalan) is higher than that of the D-isomer. The activity of the antipodes or diastereoisomers of the dipeptides also was different. One might expect that the L series amino acid derivatives would display the greatest antitumor effect; however, this was not true. For asaphan and asamet in sarcoma 45, the LL forms were most active, the DL and LD isomers were one-tenth as active, and the DD isomers were devoid of activity; for asalin on the same tumor, the DD and LD isomers were the most active, and the derivatives of L-valine (LL and DL) were less so (*97, 98*). Three and more polypeptide derivatives of alkylating amino acids were being studied.

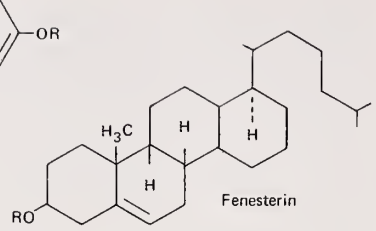
Some proposed compounds are derivatives of alkylating phenylalkanoic acids and amino acids, e.g., lophenal (4-*bis*(2-chloroethyl)aminophenylacetylphenylalanine), which has been approved for medical use, and phenamet and phentermine (analogous derivatives of methionine and tyrosine) (*88*).

The structure of the amino acid alkylating-fragment carrier apparently is important in the distribution processes of the compound in the body, active transport through membranes, catabolic decomposition processes and, possibly, in the alkylator–receptor reaction. However, the activity patterns were extremely complicated and could not be subject to unambiguous interpretation, as one might expect.

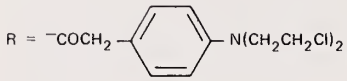
Although investigators have worked on combining alkylating and hormonal components in a molecule, some have discontinued studying derivatives of hormones having a dichloroethylamino or ethyleneimine group bonded with a C–N bond, since there was little likelihood the required biologic activity of the modified hormone molecule would be preserved (*57*). The hormone derivatives studied can be considered as depot forms. It has been hypothesized that the alkylator and hormone compound derivatives are distributed differently in the body due to the hormone component and, after hydrolysis, the hormone and alkylating effects appear simultaneously. These investigations were stimulated by the tropism discovered in certain alkylating compounds toward the endocrine glands and by the dependency of the preparations on the hormone state of animals (*99*). Alkylating derivatives of cholesterol [fenesterin (phenesterine) and butesterin], sinestrol [fenestrol (phenestrol)], diethylstilbestrol, and others were obtained, as well as the dichloroethylhydrazones of some steroid hormones. The effect of these preparations on hormone-dependent tumors and on those sensitive to alkylating agents is being studied at IECO, UPI, Leningrad Chemical–Pharmaceutical Institute, Scientific Research Institute of Endocrinology and Hormone Chemistry in Kharkov (*69, 90, 95*).



Fenestrol



Fenesterin

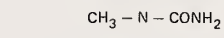


Scientists at the Institute of Organic Chemistry, Siberian Section of the U.S.S.R. Academy of Sciences in Novosibirsk, are performing interesting work on the molecular biologic bases of action of alkylating compounds. Syntheses have been accomplished and studies are being done on the properties of "complementarily addressed" reagents for alkylation of nucleic acids and alkylating derivatives of oligonucleotides [by the oligonucleotide portion of the molecule forming a complex with the complementary nucleotide sequence in the nucleic acid and then specifically alkylating the components of this acid next to the complex, i.e., modifying a selected link of the macromolecule (*8, 34*)].

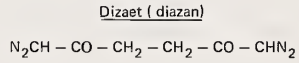
One of the most fascinating areas of research is that being done on a new type of alkylator. Study of alkylnitrosourea at the Institute of Chemical Physics, U.S.S.R. Academy of Sciences, was begun with data on the mutagenic activity of these compounds that I. A. Rapoport acquired in 1947. Methylnitrosourea and its analogues have a double action, alkylating and carbamoylating, in which they act on DNA bases and RNA polymerase, suppress the early stages of transcription and translation, bind to the protein regulatory factors and, moreover, damage the protein-synthesizing system of the cell and the NAD system. Work was done on a series of alkyl derivatives of nitrosourea. The research was successful and methylnitrosourea, distinguished by biologic effects from the American compounds of this series (BCNU, CCNU, and others) was made available for clinical study (*32, 33, 67*). Diazan, a representative of the related diazoalkanes, also was approved for testing in the clinic (*26, 47, 48*).

Some of our scientists have performed research on diazoacetyl derivatives of amino acids (*6*) and other natural and synthetic compounds, which are diazoalkane derivatives.

Methylnitrosourea



Dizaet (diazan)



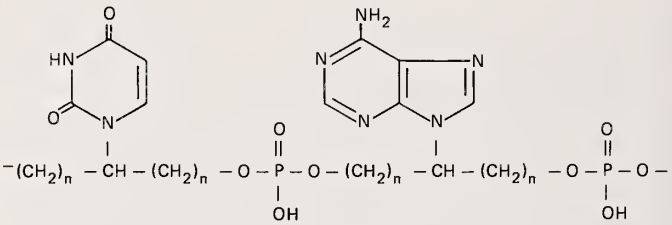
COMPOUNDS BINDING TO NUCLEIC ACIDS AND AFFECTING THEIR FUNCTIONS

For their antitumor effect, many compounds depend on their ability to bind to nucleic acids; covalent bonds form by means of intercalation and other actions. By preventing the replication and transcription of translation processes, these compounds can be classed as antibiotics. They are considered in the review that follows.

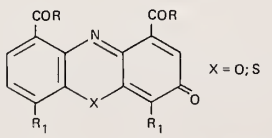
In the IOS, Latvian S.S.R. Academy of Sciences, synthesis of oligonucleotides and polynucleotides has been accomplished, and work is progressing on model analogues having conformations close to those of natural nucleic acids and capable of becoming complementary to the responsible cancer cell operon, in sequence with the purine and pyrimidine bases. The idea was suggested that similar polymer analogues of nucleic acids might be gene repressors or activators; they are more stable than the analogous polynucleotides to the action of different types of degradation enzymes (*71*). Model analogues of the oligonucleotides and polynucleotides (in particular, the analogue of polythymidylic acid) slow down RNA synthesis on a number of DNA matrices by the action of DNA-dependent RNA polymerase; they also affect protein synthesis in the noncellular system (*39*). As interferonogens, these polymers attracted our interest.

A number of our researchers are working on synthetic and natural interferonogens, in particular, the double-stranded poly I:poly C complex [Institute of High Molecular Weight Compounds, U.S.S.R. Academy of Sciences, Leningrad (*4, 22*)].

Synthetic analogues of actinomycin, which is capable of reacting with DNA, are being investigated at the LTI. A change in optical properties of DNA when the compound is added and evidence of the formation of a DNA–actinomycin analogue complex are selection criteria (*29*). Some of the resulting compounds inhibit DNA-dependent RNA synthesis at the same concentrations as actinomycin.



Starting with compounds in which the amino acid residue is bound to the nucleic base, scientists synthesized previously unknown oligonucleopeptides that react with RNA (*59, 71*). The synthesis of histone analogues should also be mentioned because some of them have the ability to suppress genetic activity. With this background work accomplished, study of different types of analogues, e.g., those containing an amino acid with an alkylating fragment or a nonalkylating, potential antimetabolite of the natural amino acid was begun. The synthetic analogues of protamines were also investigated (*87*).



ANALOGUES OF NUCLEIC ACID COMPONENTS

Antimetabolites of nucleic acids are of great interest to the scientific community because they are excellent sources in research on normal and tumor cell metabolism and in the development of new antitumor and antiviral agents. Because of these antimetabolites, certain molecular biology concepts that form the basis by which highly effective compounds are synthesized could be extended; however, further expansion of biochemical and molecular biology is necessary if new products are to be realized.

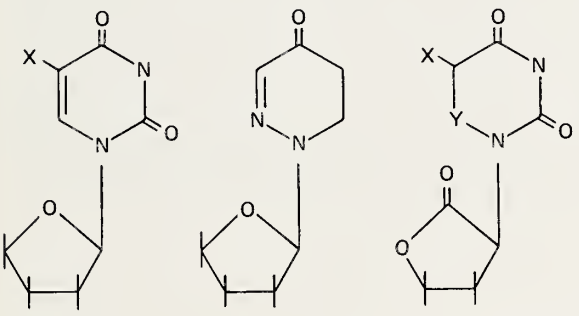
At the IOS, Latvian S.S.R. Academy of Sciences, compounds of the 1-furanidylpyrimidine and 1-pyranidylpyrimidine classes have been studied. Racemic derivatives of 5-fluorouracil (5-FU), 5-methyluracil, 5-trifluoromethyluracil, 6-azauracil, and other substituted uracils have been obtained; derivatives of substituted uracils and butyrolactone have been studied.

Ftorafur [1-(2-furanidyl)-5-fluorouracil], which has a better therapeutic index than fluorouracil, was approved for use in the treatment of malignant tumors of the gastrointestinal tract, breast, and certain other tissues (*27*). The mechanism of action of ftorafur and how it is activated is now under study, but we do know that it forms fluorouracil and the products of its metabolism in man and animals. The compound determines the possibility of prolonged circulation of these substances in the bloodstream. Distribution of ftorafur and its metabolites in the tissues differs significantly from that of substances formed after administration of 5-FU. The hypothesis has been expressed, with which many of the experimenters agree, that ftorafur is converted into 5-FU and the tetrahydrofuran derivative (perhaps oxytetrahydrofuran or butyrolactone) by the action of oxidases. An interesting model, 1-(2-furanidyl)-5-trifluoromethyluracil, has antitumor activity experimentally (British Patent No. 1,168,391; U.S.A. Patent No. 3,635,946; *41*). 5-Trifluorothymine has little activity, since it is not a substrate for an enzyme capable of converting it into an analogue of the nucleotide. Trifluorothymidine-1-2′-deoxy-β-D-ribofuranosyl-5-trifluoromethyluracil phosphate displays antitumor activity by inhibiting thymidylate synthetase. Study of the activation mechanism of the trifluoromethyl analogue of ftorafur is assisting us to understand the mechanism of action of ftorafur. It is possible that the steric similarity of the furanidyl and deoxyribofuranoside fragments have a specific function in the biochemical conversion of these compounds. Antimetabolite activity was demonstrated for the corresponding 5-substituted pyrimidine derivatives of butyrolactone (*40, 71*).

A search for the antimetabolites of nucleic acid metabolism was made among the pyrimidyl and purinyl amino acids (IOS). N^1-pyrimidyl amino acid (β-(uracilyl-N^1)-α-alanine and its 5- derivatives) apparently are of the greatest interest. N^9-purinyl amino acid has been synthesized (*60, 73*).

Amino acid derivatives of cytosine at the exocyclic nitrogen atom were also produced (*71*). N^1-pyrimidyl- and N^9-purinyl-alkanediols may be potential inhibitors of enzymes with nucleosides as substrates (*38, 39*).

At the UPI in Sverdlovsk, investigators are synthesizing and studying conversion of analogues of imidazole precursors of the purines, structures related to aminoimidazolecarboxamide (*72*). A large series of aminoimidazolecarboxamide analogues has been produced at the VNIKhFI (*30*).

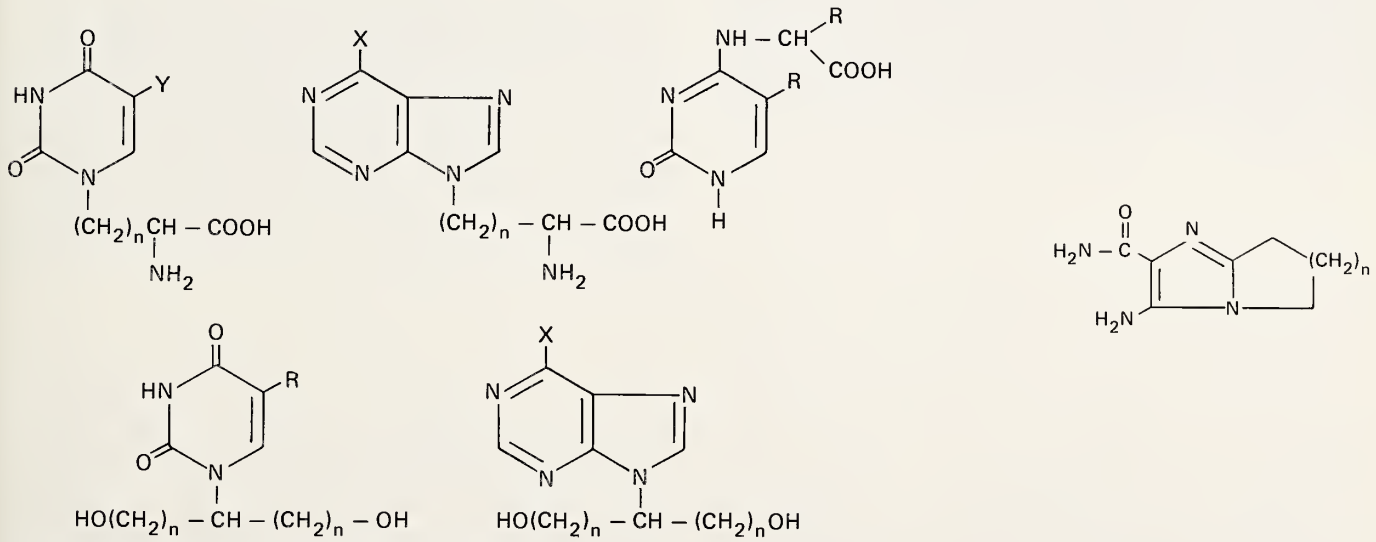


X = F — Ftorafur

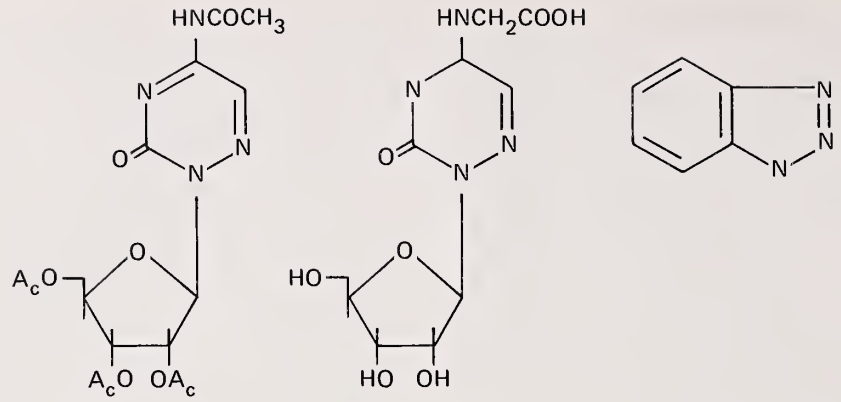
X = H, CH_3, CF_3, Br, and others

X = H, F, CH_3 and others

Y = CH, N



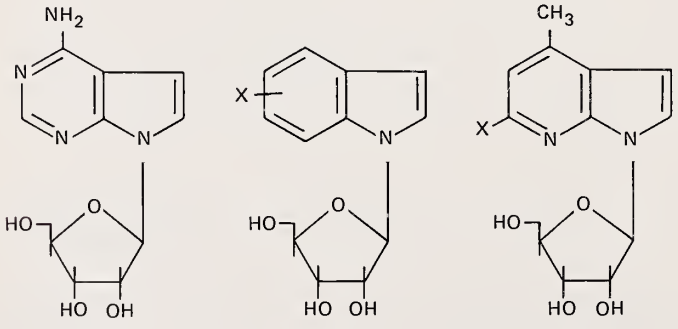
X=H,F,CH_3, and others. Y=CH,N. R=$CONH_2$, COOR.



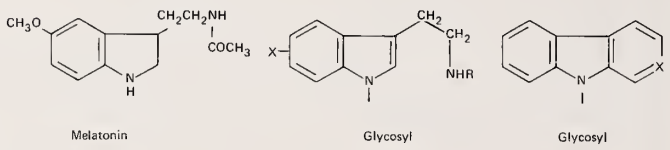
At the Institute of Molecular Biology and Genetics, Ukrainian S.S.R. Academy of Sciences, work is progressing on anomalous nucleosides, which are glycosides of 6-azapyrimidine derivatives, benztriazole, and their amino acid and ethyleneimine derivatives. Combined administration of several low-activity potential antimetabolites of the purines and pyrimidines leads occasionally to potentiation of the biologic effect (*16–20*).

Investigation of β-(puryl-C_2,C_6- or C_8)-alanines and of their peptide analogues was done at the VNIKhFI (*31*).

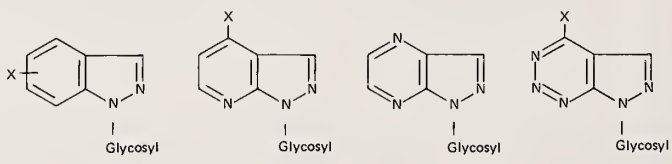
The synthesis of nucleic acid component analogues is a basic area of research in the Laboratory of Chemical Synthesis, IECO. Pyrrole analogues of purine nucleosides-*N*-glycosides of substituted indoles, pyrrolopyridines, and pyrrolopyrimidines are being studied (*77, 79*). These compounds are significant because of their relation to the biologic properties of the antibiotic tubercidin, 1-β-D-ribofuranosyl-4-aminopyrrolo(2, 3-*d*)pyrimidine (7-deazoadenosine). The corresponding base, 7-deazadenine, is not converted in the cell into the corresponding nucleoside or its phosphate, is biologically inactive, and is not deaminated by adenosinedeaminase, whereas the nucleoside analogue is a substrate of kinase, reductase, and other enzymes, because it can replace adenine derivatives in the nucleic acids and coenzymes. To some extent, the *N*-nucleosides of the pyrrole heterocyclic compounds are similar to *C*-nucleosides (which have attracted many investigators in recent years), because they cannot be degraded by phosphorylases. Some 1-glycosylindoles exhibit antitumor activity in experimental systems.



Methods have been developed for producing 1-glycosyltryptamines, which can be considered as new analogues of purine nucleosides and as new modifications of the tryptamine molecules. They are of scientific interest because melatonin binds proteins of the cell mitotic spindles in some systems; moreover, 1-glycosyltryptamines are also analogues of the purine nucleosides, which contain a fragment potentially capable of being a peptide chain acceptor (in protein biosynthesis in the ribosomes). *N*-Glycosides of harmane were synthesized; these compounds are the first representatives of indole alkaloid glycosides (*101*). Because the carbazole nucleus is the base of the structure of certain antitumor alkaloids, research on the *N*-glycosylcarbazoles was considered worthwhile (*105*).

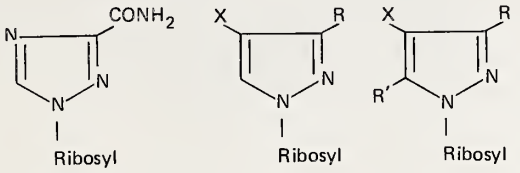


The properties of the pyrazole analogues of the purine nucleosides are being studied (*49–52*). By substitution of the imidazole ring by a pyrazole, the purine analogue remains the substrate for many purine-metabolizing enzymes and, at the same time, it becomes an inhibitor of some of them. Data are available on the antimetabolic activity of the pyrazole analogue of adenosine, and much interest has been given to 1- (and 2-) glycosides (ribosides, xylosides, and others) of the pyrazoles, condensed with various aromatic rings, e.g., benzol (indazole), pyridine, pyrazine, triazine, etc. The types of compounds studied are presented below:



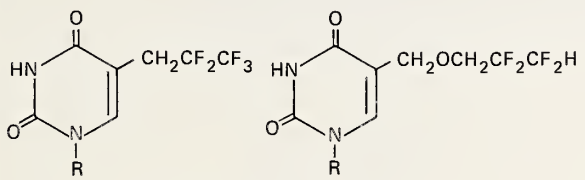
X = Condensate

Pyrazole analogues of the aminoimidazolecarboxamide riboside are being investigated (*53*), since their structure is closely related to the antiviral compound ribovirin (virazol).

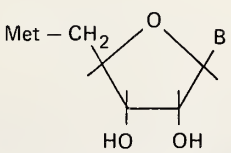


X = NO_2, NH_2
R = $CONH_2$
R' = COOR

In cooperation with the Laboratory of Fluoroorganic Compounds, workers at INEOS, U.S.S.R. Academy of Sciences, synthesized 5-polyfluoroalkylmethyluracils and 5-polyfluoroalkoxymethyluracils and their 1-glycofuranosides (*63*). With data on the structure of the active center of the enzyme thymidylate synthetase available, finding inhibitors of this enzyme among the substituted uracils containing a bulky hydrophobic substituent in position 5 was essential. It was found that 1-β-D-ribofuranosides and 1-β-D-arabinofuranosides of 5-polyfluoroalkylmethyluracils or 5-polyfluoroalkoxymethyluracils are not active in those experimental systems in which trifluorothymidine shows anticancer activity. These facts seemed to demonstrate that the high chemical lability of the C–F bond, which provides the covalent bonding of substrate analogue with thymidylate synthetase, is most important. "Two-headed" nucleosides containing a second nucleus in the core, in addition to the natural nucleic base, were synthesized, and the presence of interaction between the heterocyclic bases in these compounds was demonstrated. The compounds are important because they may be the agents that intercalate between bases of the polymer chain and act on nucleic acid conversions (*80, 102, 103*).

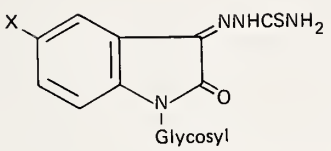


R = H, β-D-ribofuranosyl, β-D-arabinofuranosyl



B = Uracyl, 6-azauracyl, adenyl

In addition to the known antiviral activity of 1-methylisatine 3-thiosemicarbazone, the biologic properties of different 1-glycosylisatines and their thiosemicarbazones were investigated. 1-Glucopyranosylisatine thiosemicarbazones stimulate the growth of Lewis lung tumor in mice (*106, 107*).



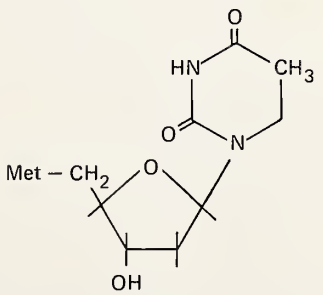
X=Condensate

Scientists at the All-Union Scientific Research Institute of Vitamins (VNIVI) have synthesized the cobalamide coenzymes 5′-deoxyadenosylcobalamine and methylcobalamine and found them strong inhibitors of the B_{12}-dependent enzymes (*109, 110*). The role of vitamin B_{12} in metabolic processes and the considerable change in B_{12} biosynthesis in certain tumor diseases must be kept in mind in the search for drugs among the analogues of the cobalamide coenzyme. Transmethylase inhibitors among the derivatives of *S*-adenosylhomocysteine have been studied, and *S*-uridyl-, *S*-cytidyl-, *S*-inosyl-, and *S*-guanosyl-L-homocysteines were synthesized (*108*).

The complex approach to finding antitumor drugs is that no differences have been found in the biochemical processes of normal and tumor cells, which might be used in control of tumors. However, an approach can be taken, that, in a cancer cell, by virtue of differentiation, there is a change in the isoenzyme composition of the major enzymes and loss of control over the tumor by the body; therefore, the selectivity of certain enzymes is lost, and the ability of reacting with compounds that are comparatively remote analogues of the natural substrates appears. A well-known example of this type of analogue is thioguanine 9-α-D-deoxyriboside, which is phosphorylated by tumor cell enzymes but is not phosphorylated in normal bone marrow cells.

The reason why even that low selectivity of existing antitumor compounds has been achieved is not completely clear. A selective distribution of the substances of their metabolites apparently is important. Unfortunately, this property is difficult to explain and even harder to predict. In this connection, the method of delivery of cytostatics to definite cells should be mentioned here. The idea is to obtain macromolecular compounds constructed according to virus types, the membrane of which

Met = 3-indolyl, 6-methylmercapto-9-purinyl

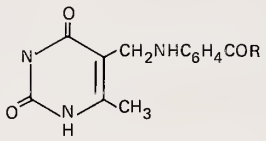


would consist of viral proteins or organ- or tissue-specific antibodies and the core of the chemotherapeutic compound bonded

covalently to the protein carrier. Mercaptopurine, cross-linked with albumin molecules and forming granules of a specific size, was used. The inner component provides the chemotherapeutic activity and the outer component supplies the tropism (*62, 96*).

SEARCH FOR POTENTIAL FOLIC ACID METABOLISM INHIBITORS

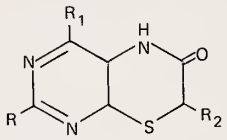
Determination of the important role of folic acid, which participates in many biosynthetic processes including those of de novo nucleoside biosynthesis, has led to a search for folic acid antimetabolites. The antifolic compounds used at the present time are usually dihydrofolate reductase inhibitors. Potential antifolic compounds are being developed at several institutions (VNIKhFI, VNIVI, IECO, UPI, Institute of Chemistry of the Ural Scientific Center of the U.S.S.R. Academy of Sciences and others), whereas at the A. N. Bach Institute of Biochemistry, scientists are conducting studies of the reactions of the substances obtained with dihydrofolate reductase (*2, 9*).



R = OH or glutamyl

Of a number of pyrimidine analogues of folic acid that have been obtained, the 2,4-derivatives of 6-methyluracil have the greatest inhibiting effect on dihydrofolate reductase (*100*).

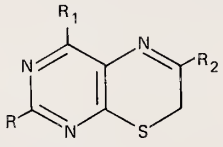
From the investigations on analogues of folic acid and aminopterin, in which the pteridine residue is replaced by a naphthyl, thienyl, or other aromatic nucleus, and those analogues in which aminophenylacetic acid has been included in the side chain in place of the aminobenzoic acid residue (*36, 83*), came the knowledge how to produce pteroyl-γ-fluoroglutamic acid (*1*). Much work on the synthesis of enzyme inhibitors of folic acid metabolism has been done at the VNIKhFI. Of the derivatives of pyrimido(4,5-b) (1,4)thiazine, pyrido(2,3-b)thiazine, and pyrazo(2,3-b)(1,4)thiazine that have been studied, 2-amino-4,6-dioxo-4-alkoxy-6-oxypyrimidothiazine strongly inhibits dihydrofolate reductase:



R = NH_2; H

R_1 = OH; ACK

R_2 = H; $OCOC_2HS$ and others



R = NH_2; H

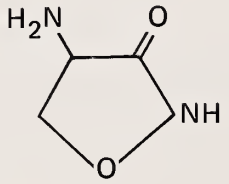
R_1 = SCH_3

R_2 = NH_2

Derivatives with an amino group in position 6 also have inhibiting properties. If there is an alkyl substituent in position 4, the inhibiting capacity is weakened or disappears. Opening the thiazine ring leads to loss of inhibiting properties. It has been proposed that the ability of substances of this class to react with dihydrofolate reductase is due to the presence of the bicyclic pyrimidothiazine system, and it is strengthened when there are reactive groups (OH, OCH_3, NH_2, etc.) in positions 4 and 6 of the bicyclic compound (*3, 21, 90, 91*). 6-Aminopyrimidothiazine inhibits not only dihydrofolate reductase but also the enzyme inactivating aminopterin, by reducing it to the dihydro and tetrahydro derivatives (*3*).

Efforts have been made in recent years to change the way the metabolite is used, so as to increase its damaging action on tumor cells and to decrease the toxic effect on the body. Selective protection from the toxicity of the antimetabolite, administered in solution, has been proposed, based on the fact that a protector-metabolite is given parenterally in the form of corpuscles or on a corpuscular carrier, selectively protecting the bone marrow, lymph nodes, liver, and spleen; by administration into the gastrointestinal tract, the intestinal epithelium is selectively protected. This principle can be used for selective effect on the corresponding organs (*11, 74*).

Vitamin B_6 antagonists have potential activity on folic acid metabolism, since one of the key enzymes of the folic acid cycle serine hydroxymethyltransferase (SOMT), essential for the biosynthesis of 5, 10-methylenetetrahydrofolic acid, is a pyridoxal phosphate-dependent enzyme. SOMT displays high resistance toward B_6 avitaminosis and to the action of diverse vitamin B_6 antagonists; however, it is inhibited by the antibiotic D-cycloserine. The activity of SOMT is increased in the tissues of patients with tumors, and the rate of biosynthesis of pyridoxal phosphate and its content in the tissues is reduced. This was the premise for studying the blocking of SOMT with pyridoxine antagonists, in particular, by D-cycloserine. To establish sufficiently high concentrations of cycloserine to ensure a chemotherapeutic effect, the highly purified compound must be used. The observed effect of cycloserine in the treatment of certain experimental leukemias is like the suppression of SOMT in the target tissues. At the present time, cycloserine (lidonomycin) is being tested clinically (*12–14*). Work on the synthesis of the potential vitamin B_2 antimetabolite isoalloxazine and its analogues is in progress (*82*).



SEARCH FOR MITOSIS INHIBITORS

New antitumor compounds among the potential mitosis inhibitors are being sought by our researchers at IECO, in the All-Union Scientific Research Institute of Medicinal Plants, U.S.S.R. MMP (Ministry of the Medicinal Industry), and by those in other institutions. Some derivatives of colchicine have been studied, and a search for alkaloids related to vinblastine and vincristine is being made among the plants growing in the U.S.S.R.

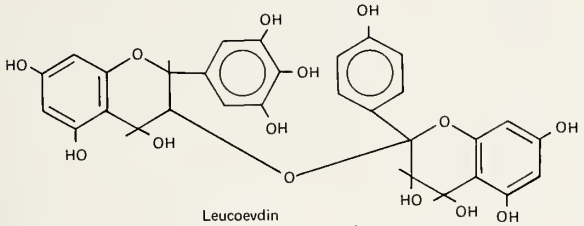
OTHER COMPOUNDS ISOLATED FROM NATURAL SOURCES

The search for antitumor compounds among natural substances of vegetable or animal origin is highly promising and is of great theoretical value, since the discovery of a compound with a new mechanism of action or with a different structure is a stimulus for development of synthetic and biologic investigations in new areas. It is precisely in living organisms that the isolation of complex biopolymers with regulatory activity can be expected.

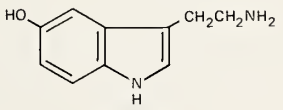
In recent years, the attention of investigators has been attracted to plant polysaccharides. The preparations chanerol (polymeric phenol) and chanerosan (a mixture of polyphenol and polysaccharide components) have been isolated from the plant *Chamaenerium angustifolium* (willow herb) at IECO (*75, 85, 86*). The anticancer properties of furocoumarins of natural origin have been studied in the VILR; peucedanin is one of the compounds of this class (*37, 70, 104*).

At the Kazakh Institute of Oncology and Radiology, scientists are studying the antitumor properties of compounds of the leucocyanidin and catechin classes, isolated from plants growing in Kazakhstan. Leucoevdin, a product of condensation of leucodelphinidin (5,7,3′,4′,5′-pentaoxyflavan-3,4-diol) and leucopelargonidin (5,7,4-trioxyflavan-3,4-diol), is being tested clinically (*105*). Antitumor activity was found in various flavone, coumarin, and tannin derivatives (*42*).

A large series of investigations were performed at IECO on the antitumor properties of serotonin and its related compounds. At a concentration of 320 μg serotonin/ml, a strong and irreversible inhibition takes place in the mitotic activity of HeLa and Ca or cell cultures. It is presumed that serotonin reversibly blocks the G_2 phase of the cell cycle. Although it displays an antitumor effect in nonsyngeneic mice with implanted tumors, serotonin had no activity in clinical tests; further study in this field appears promising (*84*).



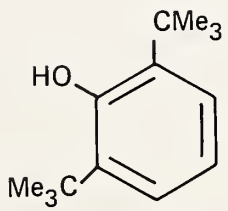
Leucoevdin
(one possible structure)



Serotonin

RADICAL REACTION INHIBITORS

In 1957, N. M. Emanuel proposed the use of low toxicity inhibitors of free radical reactions of the shielded phenol class as carcinolytic agents, based on the hypothesis of the important function free radical processes have in the development of numerous diseases. It was assumed that the action of low toxicity synthetic inhibitors compensate for a deficiency of natural (endogenous) antioxidants formed in tumor cells. Some of these compounds displayed significant antitumor activity, e.g., 4-methyl-2,6-di-tert-butyl phenol (ionol); 4-oxy-3,5-di-tert-butyl benzylamine bromhydrate, *N, N*-diethyl-(3,5-di-tert-butyl-4-oxybenzyl) amine chlorhydrate (ambunol); and the *N*-propyl ester of gallic acid (propyl gallate). These compounds inhibit the activity of dehydrases and cytochrome oxidase, suppress glycolysis, interrupt respiration and phosphorylation in the mitochondria, change the total antioxidant activity of lipids, disrupt the life cycle and mitotic activity of tumor cells, and have an anticarcinogenic effect. The possibility of affecting tumor-cell proliferation by means of natural antioxidants and synthetic radical reaction inhibitors is explained by the authors as preservation of the relationship among antioxidant activity, radical concentration, and proliferation in tumor cells (*15, 25*). Phenol compounds directly affect the transcription and translation processes in tumor cells (*55*). Ionol is used in the treatment of large papillomas of the urinary bladder.



Ionol

SUMMARY

From the standpoint of increasing the effectiveness of the search for new antitumor compounds, mutual enrichment can be attained by sharing ideas on and by cooperative work in the study of the relationships between structures and mechanisms of action of antitumor, antiviral, immunosuppressive, carcinogenic, and mutagenic substances. This work is closely bound to the leading fundamental research in the U.S.S.R. in synthetic organic chemistry, biochemistry, molecular biology, oncology, virology, and other allied disciplines, a relationship that is essential if success is to be achieved.

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Chemistry and Mechanisms of Action of Antitumor Antibiotics

M. G. Brazhnikova[1] *and* Yu V. Dudnik[2]

This paper reviews the chemical properties, structure, and mechanism of action of antitumor antibiotics produced in the Soviet Union. At the present time, the antitumor antibiotics olivomycin, bruneomycin, and rubomycin are being used by the medical profession; carminomycin is undergoing clinical trial in the treatment of various oncologic diseases.

OLIVOMYCIN

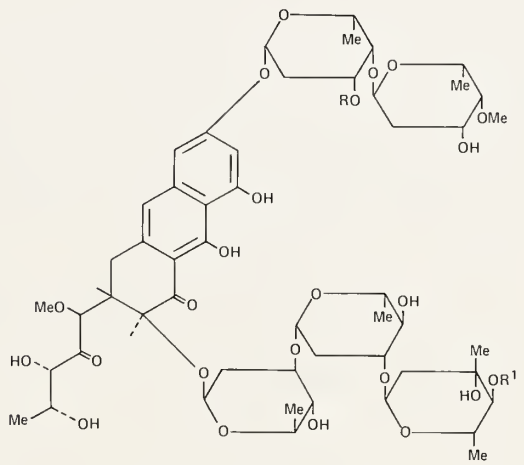
The antibiotic olivomycin, discovered at the Institute of New Antibiotics in 1962 (*19*), is formed by a culture of *Actinomyces olivoreticuli* and is isolated by organic solvent extraction of the culture liquid (*12*). This antibiotic is produced in the form of a yellow crystalline preparation, insoluble in water and petroleum ether, but highly soluble in alcohols, complex esters, chlorinated hydrocarbons, and alkalis. Olivomycin forms water-soluble salts with the latter; it is the sodium salt that is used medicinally.

Subsequent chromatography of this drug and investigation by the countercurrent distribution method has shown that it consists of three biologically active components: A, B, and C (*11*). Total preparations of the antibiotic contain olivomycin A, the most active biologically and therapeutically, in the largest quantity (up to 80%).

Olivomycin has a characteristic absorption spectrum, with maxima at 225, 275, and 405 nm. It forms acetates and the methyl ester and is split into aglycone and carbohydrate components by acid hydrolysis and methanolysis (*25, 50, 51, 54*). The aglycone, called "olivin," was purified by silica gel chromatography and was obtained in crystalline form. Study of the UV, infrared, and nuclear magnetic resonance (NMR) spectra of olivin and its derivatives (the acetates and the methyl ester), demonstrated the presence of three alcohol hydroxyls in olivin. It was determined that olivin contains the 1,8-dioxynaphthalene system, forms acetonide, and yields tetraacetylolivinic acid upon oxidation with HIO_4 of the tetraacetate of the antibiotic. The results obtained indicate that three linearly connected rings are present and that olivin is a hexaoxymethoxydiketone.

In the antibiotic, olivin is connected with five carbohydrate components, the bases of which are 2,6-dideoxyhexoses, called "oliose," "olivose," "olivomose," and "olivomycose." Study of the products of partial hydrolysis of olivomycin A revealed that the latter is the pentoside olivin, in which the 4-isobutyrylolivomycose, olivomose, and 3-acetyloliose residues and two oliose residues are connected in two unbranched chains joined to the second and sixth hydroxyls of aglycone [4-isobutyrylolivomycose and olivomose occupy the terminal position in the hydrocarbon chains (*2, 52, 53*)].

According to calculations based on Kline's law, the polarimetric characteristics of all carbohydrate components and products of partial hydrolysis of olivomycin A permitted the determination of the molecular configuration of the glycoside centers. This made it possible to conclude that olivomycin A is 2-(α-4-isobutyrylolivomycosyl)(1→3)-β-olivosyl(1→3)-β-olivosyl-6-(α-olivomosyl)(1→4)-β-3-acetyloliosyl)-olivin, i.e., it has structure I, presented in text-figure 1 (*1, 3, 4*).



Olivomycin	R	R¹
A	Ac	^{i}PrCO
B	Ac	Ac
C	H	^{i}PrCO

TEXT-FIGURE 1.—Olivomycins A, B, and C.

Olivomycin B, in distinction from the A form, contains a 4-acetylolivomycose residue in place of its 4-isobutyrate (structure II, text-fig. 1). Study of the carbohydrate composition of olivomycin C has shown that this component contains the same sugars as olivomycin A, but the oliose residue in its molecule does not have an acetyl group. Consequently, olivomycin C is deacetylolivomycin A and has structure III. Thus all three biologically active components have identical glycoside bond configurations and are distinguished only by substitutions in the 3 position of the oliose and the 4 position of the olivomycose residues.

In tests on animals, olivomycin has a high antitumor activity and a broad spectrum of antitumor action (*43*). Although it suppresses growth of gram-positive bacteria, it does not act on gram-negative bacteria or fungi. The resistance of *Escherichia coli* to olivomycin must be due to poor permeability of the cell membrane, because olivomycin acts just as effectively on *E. coli* spheroplasts as on gram-negative bacteria (*16*). The mech-

[1] Head, Division of Chemistry, Institute for the Study of New Antibiotics, Moscow, U.S.S.R.
[2] Division of Microbiology, Institute for the Study of New Antibiotics.

anism of action of olivomycin resembles that of chromomycin and mithramycin. Olivomycin selectively suppresses RNA synthesis in cultures of *Staphylococcus aureus* (*32*) and *Bacillus megatherium* (*16*), in tissue cultures (*18*), and in Ehrlich's ascites carcinoma (*15*). When the production of RNA is blocked in bacteria, protein synthesis also ceases. At the same time, even with considerable suppression of RNA synthesis in animal cells, the rate of protein synthesis does not change. Investigation of the action of olivomycin in rats (*41*) has shown that it also selectively impairs RNA synthesis.

Olivomycin forms complexes with DNA in vitro; addition of DNA to a *S. aureus* culture simultaneously with the antibiotic reverses its antibacterial action (*16*). Just like chromomycin and mithramycin, olivomycin binds to DNA only in the presence of divalent metals (*13*). It is known that olivomycin forms complexes with divalent metal ions (*12*), so that it is likely that the metal–antibiotic complex reacts with the DNA. Olivomycin binds to heat-denatured DNA much less than to native DNA, and it does not react with RNA, nucleotides, or nucleosides even in the presence of metal ions. An increase in the ionic strength of the medium does not affect formation of the antibiotic complex with DNA; however, urea hampers binding (*62*). Addition of the detergent sodium dodecyl sulfate prevents olivomycin from reacting with DNA and causes dissociation of the previously formed antibiotic–DNA complexes (*25*).

Binding of olivomycin to DNA leads to a reduction in its buoyant density in cesium salt gradients. Also, the antibiotic does not change the sedimentation constants or viscosity of DNA (*59*) or the sedimentation constants of closed circular DNA supercoils (*63*). Addition of olivomycin to DNA leads to a slight increase in its melting temperature (*30*).

The antibiotic strongly inhibits the template activity of DNA. In the DNA-dependent RNA polymerase system obtained from *E. coli* and from Ehrlich's ascites carcinoma cells, olivomycin effectively inhibits RNA synthesis (*14, 16*). In isolated liver and hepatoma nuclei of rats, olivomycin also suppresses DNA-dependent RNA synthesis, e.g., in the use of purified RNA polymerase (*34*). Olivomycin does not affect initiation but it does suppress elongation of the polyribonucleotide chains (*17*) in the RNA polymerase reaction. The antibiotic also inhibits DNA synthesis in cell-free extracts; however, DNA synthesis is more resistant to olivomycin than is RNA synthesis (*14*). Suppression of RNA synthesis in the polymerase reaction depends on the nucleotide composition of the DNA template, and it is strengthened with any increase in G and C content (*13*).

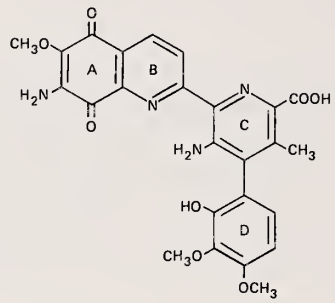
Cleavage of the glycosides from the antibiotic molecule leads to a reduction in biologic activity and to a parallel decrease in the ability to suppress the DNA-dependent RNA polymerase reaction (*30*). The olivomycin chromophore olivin does not have biologic activity, nor can it inhibit DNA-dependent RNA synthesis (*14, 30*).

Thus the biologic action of olivomycin is a result of suppression of the template activity of DNA due to the formation of the metal–antibiotic–DNA complex, which prevents passage of the RNA polymerase molecule along the template.

BRUNEOMYCIN

This antibiotic, developed in 1963 at the Institute of New Antibiotics, was isolated from a culture of *Actinomyces albus* var. *bruneomycini* (*31*) by organic solvent extraction at pH 5.0 and was purified by silica gel chromatography. The antibiotic, which crystallizes in the form of coffee-colored needles, is highly soluble in acetone, pyridine, dioxane, tetrahydrofuran, and water solutions of soda and alkalis. Although moderately soluble in ethyl acetate and chloroform, it is only slightly soluble in the lower alcohols and water. Its sodium salt is used medically. Paper and thin-layer silica gel chromatography, as well as countercurrent distribution in a Kreig apparatus, have indicated the homogeneity of this drug.

The ultraviolet and infrared spectra, elementary composition, analysis of functional groups, molecular weight, and the solubility of bruneomycin in a number of organic solvents have demonstrated the relationship of this antibiotic to streptonigrin (*13, 37*). These antibiotics have been identified by direct comparison of their physicochemical and spectral properties and their chromatographic behavior on paper (*60*).[This group of antibiotics also includes valacidin and rufochromomycin (Bromer W, McGuire YM; USA Patent No. 2,970,943, Feb. 7, 1961)]. The chemical structure of streptonigrin is presented in text-figure 2.



TEXT-FIGURE 2.—Bruneomycin (streptonigrin).

In tests on laboratory animals, bruneomycin has a high antitumor activity and inhibits the development of tumors of lymphoid origin most effectively (*44*). The antibiotic suppresses growth of both gram-positive and -negative bacteria and induces the development of temperate bacteriophages (*21*) and the production of colicin (*5*). Like many other antitumor antibiotics, bruneomycin has antimitotic and mutagenic properties, with resultant breaks and rearrangements of chromosomes (*33, 39*).

In bacterial and animal cell cultures, bruneomycin selectively suppresses DNA synthesis. A distinctive effect (which is irreversible) is that it induces severe DNA degradation; e.g., over 50% of the DNA is solubilized in 2–3 hours in *E. coli* cells. Washing the bruneomycin from the cells does not stop or decrease DNA decomposition. Suppression of protein synthesis before bruneomycin is added also does not prevent decomposition of cellular DNA (*22*). During short incubation periods, single-strand breaks are found in the DNA of bacterial cells (*61*). DNA extracted from *E. coli* cultures after a brief incubation with bruneomycin has considerably increased template activity in the DNA-dependent RNA polymerase reaction. Because RNA polymerase has a strong affinity for the 3′-OH terminals, one can assume that stimulation of template activity by bruneomycin is involved with the formation of single-strand breaks containing free 3′-OH groups (*24*). Perhaps bruneomycin activates DNA synthesis in the mitochondria of unfertilized loach roe (*57*).

Damage to DNA caused by bruneomycin apparently is repaired, with participation of the enzyme systems responsible for excision of thymine dimers and sections of DNA alkylated by mitomycin and other dual-function alkylating agents, since

impairment of these systems by mutations or by the action of trypaflavine leads to a sizable increase in bactericidal activity of bruneomycin. Upon damage to the excision system, degradation of DNA caused by bruneomycin is considerably weakened, although, in contrast to mitomycin, it does not stop completely (*26*).

The following data are evidence that bruneomycin apparently does not interact with DNA in vitro: 1) ^{3}H-Bruneomycin obtained by biosynthesis does not form complexes with isolated DNA. 2) Incubation of purified DNA with bruneomycin does not cause single-strand breaks, because of the alkaline sucrose-gradient sedimentation values that are obtained. 3) Bruneomycin does not affect RNA synthesis in the purified DNA-dependent RNA polymerase system at molar ratios of the antibiotic (nucleotide:DNA up to 18:1). 4) Finally, bruneomycin does change the nature of the melting curves of DNA at high and low ionic strengths (*24*).

Moreover, radioactive bruneomycin in intact cells binds preferentially with deoxyribonucleoprotein (DNP); 40–50% of the antibiotic is bound to the cell. The distribution of bound bruneomycin (in percent) is as follows: 43 in the nuclei of tumor cells, 15 in the mitochondria, 14 in the ribosomes, and 28 in the cytoplasm. However, a large part of the bruneomycin is weakly bound to the DNP and is released by disruption of the integrity of DNP. The strongly bound antibiotic is found mainly in the DNA fraction (*36*). Thus bruneomycin apparently reacts with structures containing DNA only after activation by the cell enzymes.

RUBOMYCIN

In the Institute of New Antibiotics, some anthracycline antibiotics that demonstrated antitumor effects in tests on animals have been obtained. One of these, tavromycetin, is a complex of antibiotics identified with cinerubin A and B, and another, rubomycin, has been studied in detail chemically and biologically.

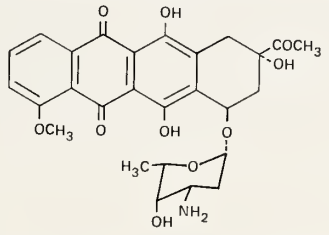
Rubomycin is contained in the mycelia and the culture medium of *Actinomyces coeruleorubidus* (*38*). Study of rubomycin by paper chromatography demonstrated that the antibiotic is nonhomogeneous and consists of a number of components designated A, B, and C (*10*). Component A, a complex mixture of pigments, has practically no biologic activity; components B and C are biologically active and show a strong antitumor effect in animal experiments.

Components B and C were synthesized in pure form by silica gel chromatography as the water-soluble hydrochloride rubomycin C hydrochloromethanol. Rubomycin B hydrochloride was obtained in the amorphous state (*6, 9*). Both antibiotics formed the red aglycone anthracyclinone and an amino sugar when hydrolyzed with hydrochloric acid. Comparison of the spectral, analytical, and chromatographic properties of rubomycin C, its aglycone, and amino sugar with daunorubicin and its aglycone daunomycinone and amino sugar daunosamine provides a concrete basis for considering that rubomycin C is identical to the antibiotics daunorubicin developed by Italian investigators and rubidomycin synthesized by the French (*46–49, 56, 57*).

There is great interest in rubomycin B, which comprises 50% of the parent preparation. This component has six times more antibacterial activity and 200 times greater toxicity in animals than does rubomycin C. Comparison of the aglycone and amino sugar isolated from the hydrolysate of rubomycin B with the aglycone and amino sugar obtained from the hydrolysate of rubomycin C has shown that both fragments are identical in each form of the antibiotic.

The following facts were obtained from our study of rubomycin B: 1) Heating or mild hydrolysis of the rubomycin B salt leads to its conversion to rubomycin C. 2) By physicochemical constants, spectral properties, biologic activity, and toxicity, this artificially produced rubomycin is identical to the biosynthetic rubomycin C. Therefore, rubomycin C is a part of the structural unit of rubomycin B. The latter upon hydrolysis loses some fragment and is converted to rubomycin C. This proposal is also confirmed by the fact that the molecular weight of rubomycin B (calculated from spectrophotometric measurements and titration data) is 120–180 units greater than that of rubomycin C. Both forms and their acetates gave different NMR spectra, but the spectrum of rubomycin B has a secondary methyl group proton signal twice the intensity of that in rubomycin C. Further investigation has shown that there is a nitrogen-free carbohydrate having reducing properties in the hydrolysate of rubomycin B after removal of the aglycone and daunosamine. Elementary analysis of this chromatographically pure carbohydrate provided the basis for our ascribing the elementary formula $C_6H_{12}O_3$ to it and for considering it to be a deoxysugar. The R_F value on paper and silica gel G in various solvent systems and the value of the specific rotation make it possible to propose that the deoxysugar is identical to rhodinose isolated from rhodomycin IV (*40*).

Thus for medical use, rubomycin C can be obtained from toxic rubomycin B, which is contained in considerable quantities in total preparations. The structural formula of daunorubicin or rubomycin C is presented in text-figure 3. In animal tests, rubomycin, a strong immunodepressant, had a broad-spectrum antitumor action and some antiviral activity (*42*).



TEXT-FIGURE 3.—Rubomycin (daunorubicin).

Rubomycin suppresses growth of gram-positive bacteria but fails to act on gram-negative ones. Resistance of *E. coli* to rubomycin must be related to the poor permeability of the cell membrane, since the antibiotic acts effectively on *E. coli* spheroplasts (*23*). Rubomycin is known to induce development of temperate bacteriophages in lysogenic bacteria (*21*). Because the antibiotic has antimitotic, mutagenic, and carcinogenic effects, one can expect various chromosomal anomalies (*33*).

In cultures of *S. aureus, Micrococcus lysodeikticus,* and *E. coli* spheroplasts, and in tumor cells, rubomycin selectively suppresses DNA synthesis (*23, 29, 32*). Moreover, DNA and RNA synthesis in *B. subtilis* cells are depressed to approximately the same extent (*23*). According to data obtained from DiMarco (*56*), the extent to which rubomycin suppresses RNA or DNA synthesis depends on the conditions in the culture.

Rubomycin forms a complex with DNA in vitro in which it binds well not only with native but also with denatured DNA (*23*). Besides reacting with RNA, synthetic polyribonucleotides, purine nucleotides, nucleosides, and bases, the antibiotic significantly stabilizes the double-helix DNA structure against the denaturing action of heat. The addition of rubomycin raises the melting temperature of DNA (*20, 27*), increases

the viscosity of DNA solutions, reduces the sedimentation constant and buoyant density of DNA in cesium density gradient centrifugation (*20, 59*), and causes unwinding of the supercoiled closed circular DNA (*63*). These properties of rubomycin–DNA complexes led to our conclusion that the planar chromophore of the antibiotic is intercalated between neighboring DNA base pairs. However, besides intercalation of the chromophore in the reaction with DNA (in stabilization of the complex), the sugar amino group of daunosamine has another important function.

The reaction of rubomycin with DNA leads to inhibition of its template activity. Rubomycin decreases DNA-dependent DNA and RNA syntheses in cell-free systems (*23, 27, 62*). Contrary to actinomycin and antibiotics of the olivomycin group, rubomycin inhibits RNA-dependent biosyntheses: RNA-dependent RNA synthesis and the reverse transcription process in the RNA-dependent DNA polymerase reaction. This property of rubomycin apparently relies on the ability of the antibiotic to react with RNA.

Rubomycin binds with DNA and synthetic polynucleotides with varying nucleotide compositions in the same way, and it suppresses their template activity with equal effectiveness (*62*). Consequently, it does not have a pronounced specificity for nucleotides. The DNA sites to which rubomycin binds most likely differ from the actinomycin binding sites. However, the reaction of rubomycin with DNA does not prevent subsequent binding of actinomycin; Dorozhinskii (*20*) found that rubomycin binds well to DNA saturated with actinomycin.

The mechanisms of action of the rubomycin B and C components are similar. Despite great differences in their biologic activity, that shown in cell-free systems differs insignificantly (*23*). Because of the presence of an additional deoxyribose radical, rubomycin B can penetrate the cells more diffusely and hence can exhibit greater activity and toxicity. Thus the biologic effect of rubomycin is one of inhibition of DNA- and RNA-dependent biosyntheses because of the antibiotic binding to the template.

CARMINOMYCIN

This new antibiotic was isolated from mycelia of the fungus *Actinomadura carminata* sp. nov. (*18*) in the form of a complex preparation that contains seven strained components, five of which have biologic activity (*7*). A powerful antitumor effect in experiments on animals was a stimulus to study this antibiotic chemically. Three of the most active carminomycins 1, 2, and 3 were separated from the inactive and from each other by organic solvent extraction and subsequent silica gel chromatography. The total preparations of the active components usually contain up to 50% carminomycin 2 and up to 30% of carminomycin 3; the carminomycin 1 content is low and in some preparations is completely absent. All active carminomycins were studied in tests on animals. From the chemotherapeutic point of view, carminomycin 1, obtained in the form of the water-soluble, crystalline hydrochloride, is of the greatest interest.

The carminomycins have ultraviolet and visible spectra characteristic of the anthracycline antibiotics. Although their spectra are closest to those of daunorubicin, they differ from the latter.

It is interesting that carminomycins 2 and 3 can be converted into carminomycin 1 by mild hydrolysis. Cleavage of a carbohydrate fragment from carminomycins 2 and 3 occurs during hydrolysis (similar to the reaction with rubomycin B). Carminomycin 1 is broken down into the red aglycone and the amino sugar carminosamine by hydrochloric acid hydrolysis. The latter was separated from the hydrolysate by chromatography on Dowex 50×12 (H+) and was obtained in crystalline form. The specific rotation of the carminosamine base and chromatography on paper and Silufol plates ascertained that carminosamine is identical to the daunosamine isolated from daunorubicin.

The *N, O*-acetates of carminosamine and daunosamine have identical NMR spectra confirming the identity of these amino sugars. The data presented indicate that carminomycin 1 differs from other antibiotics of the anthracycline group by its aglycone part, i.e., carminomycinon.

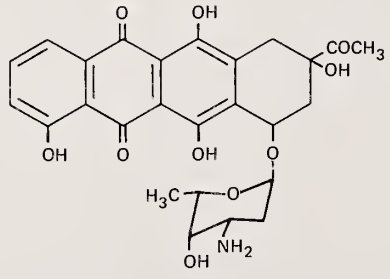
Study of the physicochemical properties of crystalline carminomycinon and its acetate and methyl esters showed that carminomycinon does not contain methoxy groups; however, the oxygen content in the carminomycin 1 molecule indicates the presence of a hydroxyl.

A comparative study of carminomycinon and daunomycinon and their derivatives disclosed a distinct difference between them. This difference is well noted in a comparison of the NMR spectra of their acetates. There is no characteristic signal of methoxy protons in the carminomycinon acetate spectrum, but there is a signal that is specific for acetyl group protons at the carbon-1 position. If the differences indicated are reliable, the methyl esters of both compounds should have identical physicochemical and spectral properties. In fact, both methyl esters have identical empirical formulas and close NMR spectra, which indicate the presence in them of four methoxy groups. The structural formula proposed for carminomycinon is confirmed by decomposition under mass spectrometric conditions. There is a molecular ion *m/e* 384 in the mass spectrum. All types of decomposition are confirmed by the presence of the corresponding metastable peaks.

The position of the glycoside bond in the antibiotic was proved by hydrogenation of the latter over palladium, i.e., hydrogenation of the glycoside bond and elimination of the hydroxyl in the aglycone take place. Hydrogenation of carminomycinon under the same conditions gives a product chromatographically identical to that obtained by hydrogenation of the antibiotic. The NMR spectrum of the acetate of the hydrogenated product showed the absence of an acetyl group at C 10, which is evidence the amino sugar is bound in the C-10 position of the aglycone (*8, 55*).

Thus carminomycin is 8-acetyl-10-(3-amino-2,3,6-trioxy-L-lyxo-hexopyranosyl)-7,8,9,10-tetrahydro-1,6,11-tetroxy-5,12-naphthacenedione. The structural formula of carminomycin 1 is presented in text-figure 4.

In tests on animals, carminomycin has a strong antitumor effect. It differs from rubomycin by greater activity toward L1210 leukemia and by none against Ehrlich's ascites carcinoma (45).



TEXT-FIGURE 4.—Carminomycin.

Carminomycin suppresses growth of gram-positive bacteria, does not affect gram-negative ones, and induces growth of lysogenic bacteria. Its mechanism of action is similar to that of rubomycin. In *M. lysodeikticus* cells, carminomycin selectively suppresses DNA synthesis and, like rubomycin, it reacts in vitro with native and denatured DNA, RNA, purine nucleotides, nucleosides, and bases. Carminomycin and rubomycin react with the polyribonucleotides poly(A), poly(AU), and poly(U), although they do not bind to free uridylic acid. Carminomycin stabilizes the double-helix structure of DNA against the denaturing effect of heat; the rise in melting temperature is higher with carminomycin than with rubomycin. Also like rubomycin, carminomycin increases the viscosity of DNA solutions. Noticeable quantitative differences in their effects are not observed. Although carminomycin suppresses the activity of the purified DNA-dependent RNA-polymerase system with native and denatured DNA as the template, there is practically no difference in the effectiveness of carminomycin and rubomycin in the use of native DNA as the template. With denatured DNA, rubomycin acts more strongly than carminomycin. Thus no fundamental differences were found in their mechanisms of action at the molecular level (*27*). Differences in the chemotherapeutic activity of these two antibiotics, just as with rubomycin B and rubomycin C, are probably due to differences in their pharmacokinetics and other actions on the body.

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Indication of Antitumor Agents by Microbiologic Methods

A. Z. Smolyanskaya[1]

The introduction of cancer chemotherapy into clinical practice and its initial successes gave added impetus to research on the chemical synthesis and isolation of active antitumor agents from natural substances. In conjunction with the research, quantitative and qualitative procedures needed to be developed and made available to scientists who would test the antitumor drugs in vitro and in vivo.

QUALITATIVE ASPECTS

Qualitative confirmation of the manifestation of antitumor activity of a compound is usually accomplished by the implantation or induction of tumors in animals. Such studies are long, expensive, laborious, and awkward; they cannot possibly encompass the entire mass of synthesized and biologically produced compounds. Recently, much attention is being given to different methods of studying antitumor activity in vitro. Various cells, tissue cultures, and microorganisms are used as test materials. The use of microorganisms is limited by the low antibacterial activity of most antitumor agents and by differences in metabolism and morphology (mainly in the cell wall) of animal and microbe cells. Nevertheless, certain microorganisms, i.e., virus particles and a number of bacterial mutants, are sensitive to antitumor compounds that act on the nucleic acids and/or protein synthesis. A number of authors have demonstrated that antitumor compounds can also affect the functional activity of various genetic structures; the capacity of these preparations to derepress the extrachromosomal genetic elements and, correspondingly, to cause colicin synthesis, transition of prophage to the vegetative form, etc., are examples (*16, 19, 20, 22, 25, 32, 34*). All antitumor antibiotics in particular have this ability. As early as the 1950's, some microbiologists reported on a correlation between antitumor activity of a number of these drugs and their ability to induce prophage of the lysogenic strain of *Escherichia coli* (*34, 39*). Later, other authors (*23, 24, 29*) stated that these antibiotics can suppress reproduction of the vegetative phage. These and some of their other studies were the basis for the development of various microbiologic test systems in which lysogenic bacteria were used in the initial screening of antitumor antibiotics.

However, for other groups of antitumor agents (alkylating compounds, antimetabolites, preparations of plant origin, etc.), microbiologic test systems based on induction of prophage of lysogenic bacteria or on the suppression of reproduction of the vegetative phage proved to be less suitable. In vitro tests to improve the sensitivity of the systems with the use of various detergents [e.g., dimethyl sulfoxide (DMSO) to increase cell permeability] were only partially successful. In assays with lysogenic *Staphylococcus* strains and DMSO as the solvent, known antitumor agents of the antimetabolite group and some of the alkylating compounds were active. These compounds had antiphage and/or phage-inducing effects (fig. 1). Two lysogenic strains of *Staphylococcus aureus* (No. 672 and 962) isolated from patients were used. Of the morphologically typical strains, they have multiple drug resistance (No. 672: Pc^R, Tc^R, Cm^R; No. 962: Pc^R, Tc^R, Sm^R, and Cm^R), are typed by phages of a standard collection, and have a number of enzymes characteristic of the pathogenic properties of *S. aureus*. A culture of *S. aureus* 83a is an indicator for vegetative phages of both strains. The antibacterial, antiphage, and phage-inducing activity of the preparations are manifested simultaneously in this system. A mixture of 18-hour cultures of both strains lysogenic and sensitive to the phage of it are added to nutrient agar in Hottinger broth with glucose (0.4%) and calcium chloride (0.02%), melted, and cooled to 45–50° C. The inoculated agar is poured into petri dishes, and holes, made with cylindrical punches or a borer, are filled with a specific concentration of the preparation being studied, which had been diluted with 50% DMSO solution. Around the holes, depending on the biologic activity of the preparation, three zones form. The first is the bacteria growth inhibition zone, the second is the vegetative phage reproduction zone (increased bacterial growth), and the third is the lysogenic culture prophage induction zone. In a favorable situation, either a large number of phage plaques or complete lysis of the indicator culture is noted in the third zone. Such a distribution corresponds to change in concentration of the preparation in proportion to its diffusion from the hole in the agar. The appearance of antiphage and phage-inducing zones indicates the possibility that antitumor activity will be detected in the preparation under study. By the proposed test system, new active preparations were selected, not only from the culture fluids of actinomycetes (antibiotics) but from extracts of medicinal herbs, chemical compounds which are analogues of natural metabolites, and some chloroethylamine derivatives of carboxylic acids.

Tests of the lysogenic *Staphyloccoci* selected by antiphage- and phage-inducing activity of the compounds in a culture of cells from an ovarian carcinoma (CaOv), in which the cytostatic effect of the compound was the result of suppression of thymidine in DNA, displayed complete coincidence of activity of the selected preparations. In animals with various transplanted tumors, an increase in effectiveness of screening was noted; i.e., among the test-selected compounds, about 60% were active. Nevertheless, the data obtained do not give a basis for the assertion that only antitumor activity is manifested in the system we propose. However, metabolic diversity of various species of bacteria, phages, and other microorganisms and the ease of producing controlled mutations in microbes establishes all the premises for several microbiologic test systems for initial screening of antitumor agents. The simplicity of this system and the speed of the response are due to the efficiency

[1] Cancer Research Center, Moscow, U.S.S.R.

of a complex of microbiologic tests for initial screening of antitumor compounds. The premises for establishing such assay systems are the diversity of existing microbiologic tests for the study of potential antitumor activity of various agents. The use of biochemical mutants of different drug-resistant variants of bacteria in bacterial screening tests should be mentioned here (*6, 7, 12, 23, 30, 38, 46*).

Thus the design of microbiologic systems for initial screening that is based on data of potential activity of various classes of compounds and the biochemical, morphologic, and other characteristics of microbe cells is promising; these data should be included in the first stage of screening antitumor compounds.

QUANTITATIVE ASPECTS

The development of efficient quantitative microbiologic methods for detecting antitumor agents in biologic substrates is based on the same principles.

For clinical and experimental use, the levels of antitumor drugs in body tissues and fluids must be determined. These data are essential for the definition and/or detection of toxic and therapeutic doses, for the determination of optimum routes and intervals for the administration of a drug and its pharmacokinetics, as well as for the discovery of the specific mechanisms of action each drug possesses. The amounts of a compound retained and excreted by the body are also important in treating cancer patients. Because chemical, physical, and physicochemical testing procedures are complicated and low in sensitivity, their use is limited. The most sensitive radiometric methods of quantitative analysis in vivo affect the drug, the site of administration, and the amount of label (in the compound or in the products of its catabolism).

Quantitative microbiologic methods of antitumor agents are still not sufficiently widespread. This can be explained by the low antibacterial activity of most antitumor agents already mentioned (except the antitumor antibiotics). According to the data of some of our authors, only 23–46% (depending on the test microbe) of the compounds having antitumor activity in animal experiments suppressed growth of *E. coli, Lactobacillus casei,* or *Tetrahymena pyriformis,* microbes which are the most sensitive to antitumor agents.

Antineoplastic compounds have marked DNA and/or RNA tropism or the ability to suppress protein synthesis at various stages, which is the basis of their biologic activity. The ability of most antitumor agents to act on the synthesis of proteins and nucleic acids extends to microbe cells, but sometimes it is manifested only under conditions of auxotrophism of microbes for the same metabolites that the preparations antagonize and of restriction on the number of these metabolites in the medium.

It has been shown in a number of works that, under specific conditions, antitumor agents have a significant effect on reproduction of bacteria (*5, 9, 19, 31, 37*). Practically, the appearance of each new antineoplastic compound is accompanied by extensive study of its antimicrobe spectrum and of the interactions between bacteria and antitumor compounds which can be used for the development of quantitative microbiologic testing of the latter.

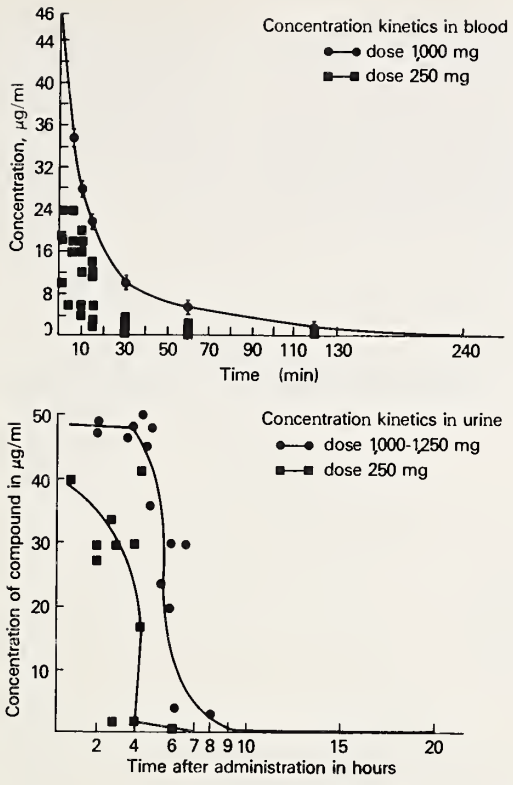
Numerous antibiotics used and proposed as antitumor agents have a definite, noticeable antibacterial activity, on which, in addition to microbiologic tests, various assays of their pharmacodynamics are based. The selection of a test microbe is made according to preliminary testing of the sensitivity of various species and strains of bacteria to a specific compound. Because some bacterial strains show sensitivity to many drugs, they are used as test microbes; e.g., spore suspensions of *Bacillus mycoides* and *B. subtilis* are used for the determination of concentrations of olivomycin, chrysomallin, bruneomycin, sibiromycin, and carminomycin (*1, 2, 8, 14, 15, 21*).

For antitumor antibiotics having a more pronounced antibacterial effect (e.g., mitomycin C, dactinomycin, or adriamycin), other bacterial species can be used: *E. coli* and *Sarcina lutea.* Nevertheless, the sensitivity of most bacteria species to antitumor antibiotics will frequently be insufficient (even when it amounts to 10^{-1}–10^{-2} μg/ml) to determine the concentration of the compound in biologic substrates from patients. However, the high toxicity of antitumor antibiotics requires that minute doses of the compounds be prescribed to patients. Thus carminomycin is used at a dose of 5 mg every other day; the single dose of bleomycin is 1–5 mg, etc. Naturally, even with iv administration of such doses, the concentrations of the antibiotics in the blood and organs of patients will be below or equal to the minimum suppressing concentration of the compound in the first minutes after administration. The minimum suppressing concentration for most antitumor antibiotics, with respect to spore cultures, are 0.1 μg/ml and for other bacterial species, from 10^{-1} to 10^{-2} μg/ml. Because animals can be given larger doses per kilogram body weight or per square meter body surface area than can be given to humans, some useful data are available on the pharmacokinetics of these drugs as a result of microbiologic testing.

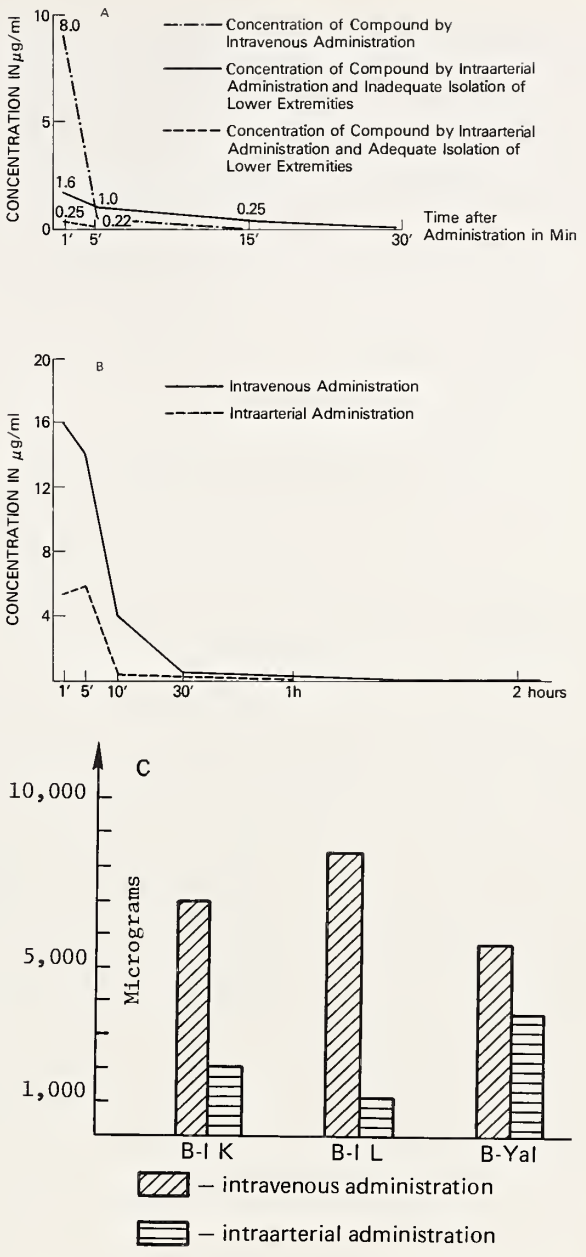
Antibiotics and other antitumor agents, particularly the antitumor antimetabolites, can act on microbe cells. However, this effect can now be manifested only under specific and rigid conditions that provide for a choice of a microbe mutant auxotrophic for those metabolites (or their precursors), which the preparation antagonizes and with precise dosage in a medium meeting the requirements of the strain. Under such conditions, bacteriostatic or bactericidal effects of a preparation can be detected as well as its capacity to change the activity of various genetic systems of the microbe cells. Among the mutants of bacteria used for these purposes, the drug-resistant ones occupy a special place. In a number of works, different resistant bacteria variants were used that proved to be more sensitive to the effect of antitumor agents than were the initial variants. Consequently, the choice of the appropriate test microbe and its culture conditions create prerequisites for the establishment of microbiologic methods of quantitative determination of most, if not all, antitumor compounds in biologic substrates. Of course, the conditions necessary for correct testing of compounds in microbiologic systems should be observed.

ELABORATION OF TEST SYSTEM FOR DETERMINATION OF 5-FLUOROURACIL CONCENTRATION IN BIOLOGIC SUBSTRATES

In preliminary in vitro tests, it was shown that bacteria of the genus *Enterococci* are the most sensitive to 5-fluorouracil (5-FU) in medium containing only the nutrient and growth substances. Their sensitivity to 5-FU is determined by their auxotrophism on folic acid, which participates directly in pyrimidine (thymidine) metabolism. As is well known, 5-FU disrupts the synthesis of the latter, which results in a blocking of thymidylate synthetase (which folic acid needs to synthesize thymidine). The strain *Streptococcus faecalis* 543, which we isolated in the clinic, is auxotrophic for a number of substances participating in pyrimidine metabolism (folic acid, calcium pantothenate, riboflavin, and tryptophan) in a medium restricted to these metabolites and to other nutrient and growth substances. This strain demonstrated a high sensitivity to 5-FU. The zone of growth retardation of the test microbe was obtained from a dose of 0.006 μg/ml. Limitation of the medium



TEXT-FIGURE 1.—Kinetic curves of 5-FU concentration in blood and urine of patients.



TEXT-FIGURE 2.—Kinetic curves of drug concentration when 5-FU is given iv, intra-arterially, and by infusion. A) In blood of patient with cancer of the colon; B) in serum of patient with breast cancer; C) in urine of patient with cancer of the pancreas (drug was administered by infusion of the organ).

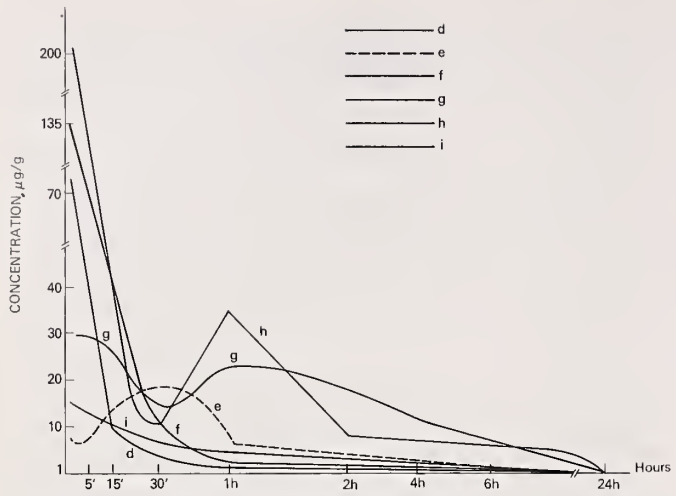
to nutrient and growth substances was achieved by dilution of the meat infusion (3, 5, or 7 times) and by less peptone added to the medium, depending on the grade, time of preparation, etc. (from 1 to 0.033%). From 1.5 to 1.7% of purified agar was added to the meat–peptone broth. The resulting thick nutrient medium was used for determination of the growth retardation zone of the test microbe by the agar diffusion method; standard microbiologic testing conditions were observed.

The range of working limits of the system was wide. On a straight segment of a curve in semilogarithmic coordinates and reflecting the dependence of the test microbe growth retardation zone on the concentration of the compound, the value of concentrations from 0.1 to 1.0 μg/ml were drawn. Additional tests showed that dilution of the preparation in the blood serum and urine did not change the trend and slope of the curve. Consequently, the system proved suitable for the determination of 5-FU concentrations in fluids from patients (*20*).

With this test system, kinetic determinations were made of the drug concentrations in the blood and the urine of patients treated with various doses and by different routes of administration. The data obtained permitted monitoring of the effectiveness and toxicity of doses, the correctness of use of the regional infusion technique, and the speed with which the drug was eliminated from the body. Blood and urine concentrations of 5-FU given iv to patients at 250- and 1,000-mg doses are presented in text-figure 1. The total amount eliminated in the urine was 3–7% of the dose administered.

In patients suffering from breast or colon cancer, the kinetic curves of concentration by iv (systemic) and intra-arterial (regional) administration of identical doses of the preparation were compared. The resulting data showed that, with a correct regional infusion technique, the concentration of the compound in the blood and its detection time decreased considerably (text-fig. 2A, B). This led to a reduction in the overall toxic reaction, which permitted the total course dose of 5-FU to be increased to 10–15 g rather than 3–5 g. We obtained similar data in a study of the concentration of the preparation by an infusion of the pancreas. As the concentration of the 5-FU circulating in the blood decreases, so does the amount excreted in the urine (text-fig. 2C).

Therefore, we consider our quantitative microbiologic methods of testing 5-FU are beneficial and important in clinical and experimental tumor chemotherapy.



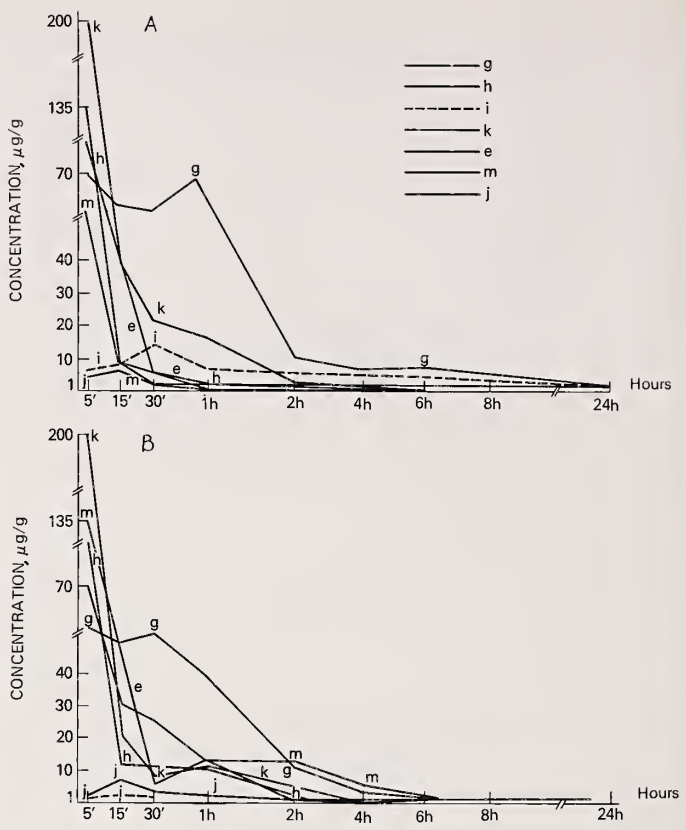
TEXT-FIGURE 3.—Distribution of 5-FU in blood and organs of intact animals. Key: d=blood; e=liver; f=lungs; g=spleen; h=kidneys; and i=brain.

DEVELOPMENT OF QUANTITATIVE MICROBIOLOGIC METHOD OF DETERMINATION OF METHOTREXATE

Similar principles were the basis for the development of a system for the quantitative determination of folic acid antagonists aminopterin and methotrexate (MTX). These compounds are direct antagonists of pteroylglutamic acid; however, the system for determination of the 5-FU concentration was insufficiently sensitive for them, although the *S. faecalis* strain 543 was a strict auxotroph for folic acid and, in the medium used, the amount of nutrient and growth substances was restricted by dilution of the meat infusion. The first such test system for MTX was proposed by J. Burchenal et al. in 1951 (*27*); the *S. faecalis* strain 8043, having high sensitivity to MTX in Difco medium, was used in the tests they developed for the determination of the folic acid content. The method of diffusion in agar from paper disks was used.

By repeated purification of the activated center of acid casein hydrolyzer, we obtained a medium with a high level of amines and total nitrogen but no folic acid and other essential microbe cell metabolites. A mutant of American Type Culture Collection *S. faecalis* strain 8043, auxotrophic for not only pteroylglutamic acid, tryptophan, riboflavin, and calcium pantothenate, but also for L-cysteine, guanine, and nicotinic acid (distinct from strain 543) was used as the test microbe. By addition of proportioned amounts of these substances to the medium indicated above, we obtained a culture in which the sensitivity of the strain to MTX was 0.0006 μg/ml, and the range of concentrations measured with a high degree of accuracy (working limits of the method) was from 0.001 to 0.01 μg/ml.

The microbiologic testing proved completely suitable for determination of 5-FU concentration in organs and tissues of experimental animals, after a method of eluting the compound from organ tissue homogenates was developed. Data were obtained on the distribution of 5-FU in controls and those animals bearing tumors differing in sensitivity to the compound (text-figs. 3–5). The concentrations of 5-FU in various organs depended on the metabolic activity of the tissue. Thus in the first minutes after administration of the maximum tolerable dose (120 mg/kg), the highest levels were found in the liver, blood, and lungs. At this time, 5-FU was detected in minimum

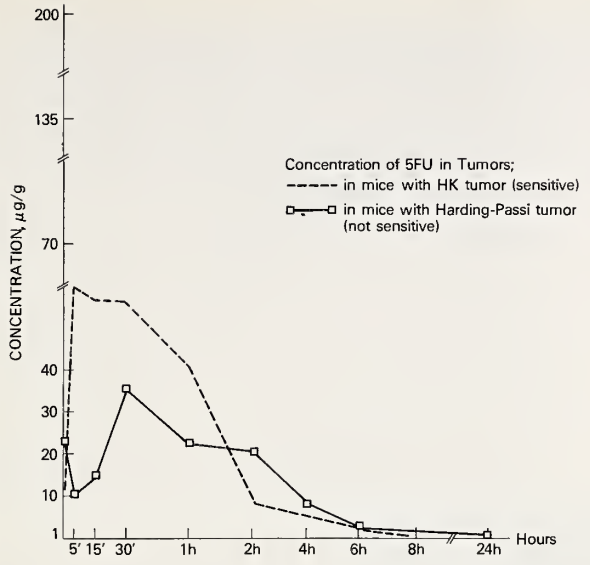


TEXT-FIGURE 4.—A) Distribution of 5-FU in organs of AK-755 tumor-bearing mice after iv injection of 120 mg/kg. B) Distribution of 5-FU in organs of mice bearing HK tumor (sensitive) after iv injection of 120 mg/kg. Key: g=tumor; h=blood; i=liver; j=brain; k=kidneys; l=lungs; m=spleen.

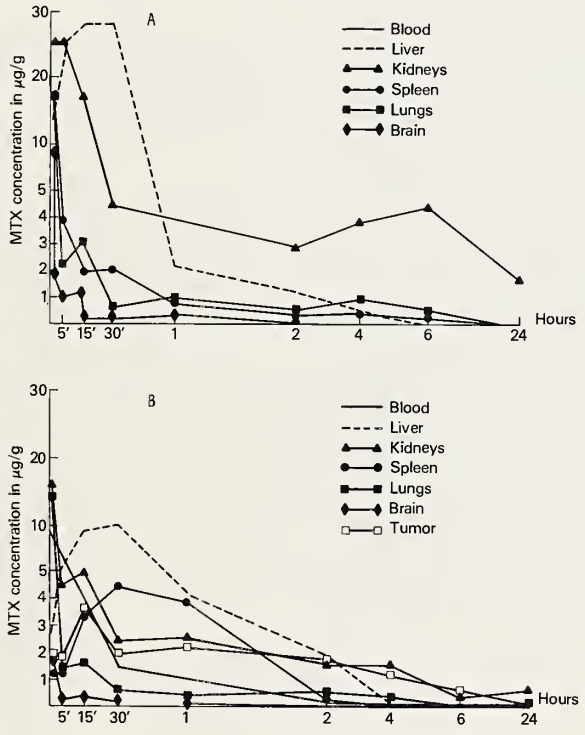
amounts in the liver and was not found at all in a number of cases. This is related to the ability of liver cells to inactivate the compound. Tissues of a sensitive tumor had a similar capacity for disintegrating 5-FU. This inactivating effect is expressed considerably less in tissues of an insensitive tumor (text-fig. 5). The concentration of the compound in the blood and organs of intact animals apparently is higher than in those with a sensitive tumor.

By this system, determination of MTX concentrations in the blood and urine of patients was accomplished, and the distribution of these compounds in the organs and tissues of animals with and without tumors was ascertained. The data obtained in these determinations indicate a similarity in the nature of distribution of MTX and 5-FU in patients and experimental animals and also permit observation of the specificities of MTX distribution. Thus in the study of MTX distribution in patients when it was given iv, specific concentrations of the compound were found in the cerebrospinal fluid; similar data were obtained in animals. Small, but quite perceptible, concentrations of MTX were found in the brains of healthy and tumor-bearing animals 1 hour after administration (text-fig. 6). As in tests with 5-FU, the concentration of MTX in animals with tumors sensitive to it was considerably lower in all organs (except the brain) than in the healthy ones.

The time during which MTX is detected in the body does not differ as significantly. During the first 24 hours, the compound is detected in all organs (except the liver) in healthy and tumor-bearing animals. However, previous administra-



TEXT-FIGURE 5.—Kinetic curves of 5-FU concentration in sensitive and insensitive tumors.



TEXT-FIGURE 6.—Kinetic curves of MTX levels in blood and organs of intact mice and those with tumors sensitive to the compound. A) Intact mice after iv injection of 20 mg/kg; B) mice with SMSh tumor after iv injection of 20 mg/kg.

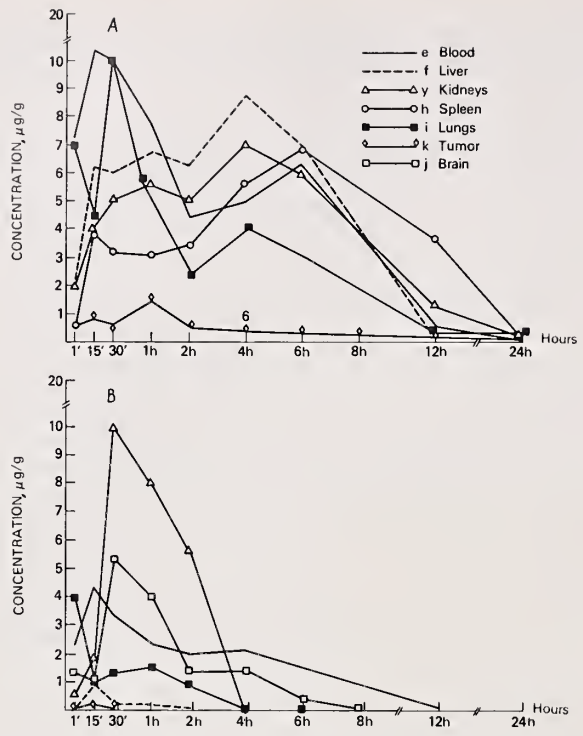
tion of leucoverin (an antidote) sharply changed the distribution of MTX: It never appeared in the bone marrow, nor was there any in most organs 4–6 hours after administration. In 30 minutes to 2 hours afterward, the MTX concentration increased considerably in the liver, kidneys, and spleen, a phenomenon that can be explained by a reduction in de novo synthesis of folate reductase, which is due to the presence of the folinic acid. The amount of enzyme that binds the MTX entering the tissues decreases. The level of MTX in the blood, lungs, and brains in the first 2 hours after administration is equivalent in tumor-bearing animals with and without the leucoverin supplement.

Although it is evident from the examples presented that microbiologic tests make possible the development and detailed study of many distinctive qualities of drug distribution in the body, this does not exhaust their importance in clinical and experimental tumor therapy. The pharmacokinetics of compounds should have extensive use.

At times, microbiologic tests can assist in the development in vitro of the biologic activity and concentrations of those preparations that become active only when administered to a living organism. Fluorafur (ftorafur), one of the new, heterocyclic antitumor compounds, which is an antimetabolite of natural nucleosides, has many features in common (e.g., chemical structures and biologic and antiblastic properties) with the antitumor antimetabolite 5-FU (*13*). This knowledge served as the basis for qualitative and quantitative determinations of a compound in biologic substrates with the aid of test system I developed for 5-FU. In preliminary tests, the activity of the compound (determined spectrophotometrically) in this system corresponded to the content of impurities in 5-FU. However, the chemotherapeutic activity of fluorafur in tests on animals and in the clinic was considerably higher than that which might have been caused by the 5-FU admixture. In a determination of the 5-FU levels in serum and organ tissues of experimental animals, concentrations of the compound were also found that exceeded those possible by administration of the same amounts of the drug, which were caused by admixture of the latter in fluorafur. Therefore, we proposed that molecules of 5-FU are split from fluorafur in the body and form concentrations higher than those resulting from the admixture. This proposal was confirmed by the studies of Merein and Belousova (*18*).

To increase the sensitivity of the microbiologic method in determining small concentrations of 5-FU that can form in animals during treatment, we used a second test system (II) with *S. faecalis* 8043 and the appropriate medium. The sensitivity of the test microbe to 5-FU in this system was five to ten times higher than in the first, but the absolute concentrations obtained in vitro in a parallel determination differed by less than 10%. Therefore, the concentration of the active compound in the blood and organs of the animals also was assayed in parallel in the two test systems. The result obtained showed that the level of the active compound revealed by the two assays differ considerably from one another. This is evidence that the biologically active part of fluorafur revealed in system II can vary from that revealed in system I not only quantitatively but qualitatively. The data suggest that fluorafur in a living organism is capable of forming a minimum of two biologically active compounds, one of which is 5-FU and the other, a different active principle having an antibacterial effect in system II and considerable antitumor activity. Actually, the serum of mice 15–20 minutes after administration of fluorafur had antiphage- and phage-inducing activity in the lysogenic *Staphylococci,* which we proposed as a test for initial selection of antitumor antibiotics and antimetabolites. Neither fluorafur nor 5-FU, in the concentrations which can form in the body, had such a capacity (*44*). These results supplied us with data to do a study on the pharmacokinetics of the active components of fluorafur in intact and tumor-bearing animals (text-fig. 7A, B).

Data acquired in these experiments indicate that a gradual transformation of the active components of fluorafur takes place in the organism. In the first 30 minutes, some of the active



TEXT-FIGURE 7.—Kinetic curves of fluorafur distribution in organs of intact (A) and tumor-bearing (B) mice.

components of fluorafur increase; then in the next 6 hours, their levels become well stabilized. However, the intact animal maintains a residual concentration of the active drug for more than 12 hours. In animals with sensitive tumors, the concentration of the active preparation is lower in the blood and organs, and the period during which it can be detected is briefer than in the intact mice (text-fig. 7A, B).

However, this does not limit the possibility of testing antitumor compounds by microbiologic methods. Thus the synergistic effect of combinations of known antitumor compounds and culture fluids was determined with microbiologic models (*4*); we used the lysogenic *Staphylococcus*. The synergistic effect of compounds in combination in animal tests was detected by antiphage- and/or phage-inducing effects of the serum of treated animals.

In this way, study of the specific interactions of antitumor compounds with microbes with the numerous biochemical structures of living organisms is important, not only for judging the mechanisms of action of antitumor chemotherapeutic compounds but also for their quantitative and qualitative indication in biologic substrates, as well as for obtaining other valuable information for tumor chemotherapy.

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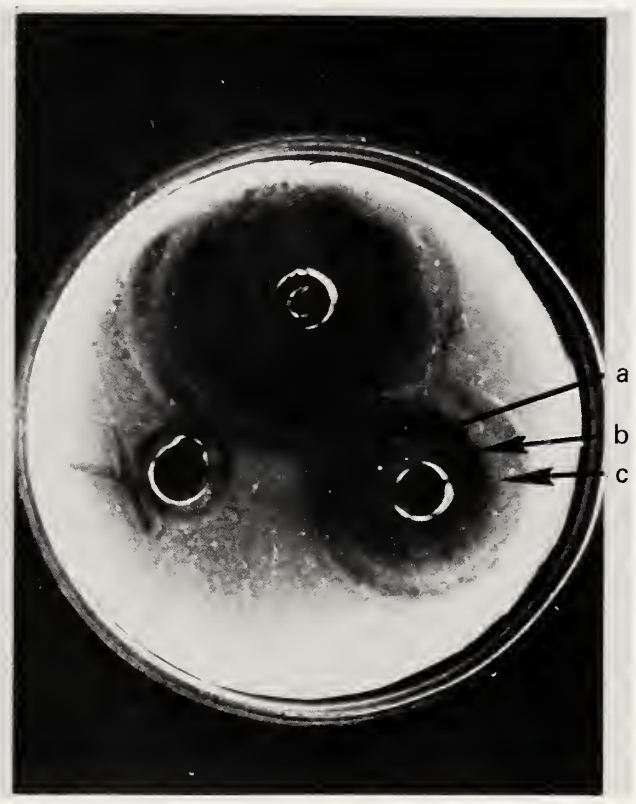

Figure 1.—Test system for developing antibacterial, antiphage, and phage-induced activity of preparations; investigation of various doses of methotrexate (MTX); a = antibacterial zone; b = antiphage zone; and c = zone of phage induction.

Methods of Screening Antitumor Antibiotics

G. F. Gauze[1] *and* V. A. Shorin[2]

The question of the extent to which experimental test systems permit correct conclusions to be drawn for clinical chemotherapy is among the important problems in the search for new drugs. In the light of this problem, we discuss here in vitro methods of screening antitumor antibiotics with various microbiologic systems and tumor cells and in vivo tests on laboratory animals.

IN VITRO SCREENING

In discussing this problem, the singularities of anticancer antibiotics now used in clinical practice should be taken into account. Rubomycin (identical with daunorubicin), bruneomycin (identical to streptonigrin), and olivomycin, which is similar to mithramycin and chromomycin in chemical structure, are used in the U.S.S.R. Sibiromycin also has a strong antitumor action in tests on animals. The first three antibiotics mentioned above can be found (by bacterial tests) in the culture fluids of the microorganisms forming them. The fourth antibiotic cannot be found by these methods, since sibiromycin is contained in the culture fluid at low concentrations, at which it suppresses only the growth of tumor cells but not the growth of bacteria. However, it was determined that at higher concentrations, this antibiotic suppresses growth of various bacteria, the hay bacillus in particular.

We have now reached the first problem in the discussion, i.e., an estimate of the significance in in vitro assays of tumor cell models in comparison with those of bacteria cells for detecting anticancer substances among the natural compounds. In our laboratory, Ivanitskaia and Makukho (*1*) investigated in detail over 1,300 actinomycete cultures and established that cultures in which tumor cell reproduction was suppressed contained antibiotics known to suppress growth of these same cells in 58% of the laboratory animals being tested. Moreover, cultures that suppressed bacterial cell growth and were inactive against tumor cells were effective in only 5% of the animals tested. It is evident from this example that the results obtained with tumor cells have considerably greater prognostic value than those with bacterial cells.

The data compiled by Ivanitskaia and Makukho did not include corrections for specific mechanisms of action. Now it is readily possible to ascertain early in our experimentation whether the test compound is a selective inhibitor of nucleic acid synthesis, an inhibitor of protein synthesis, or if it has some other mechanism of action.

Kochetkova et al. (*2*) recently demonstrated in our laboratory that information on the mechanism of action in the early stages considerably improves the prognostic value of models using bacterial cells. It is well known that all anticancer antibiotics prescribed in clinical practice (dactinomycin, mitomycin C, rubomycin, adriamycin, carminomycin, bruneomycin, olivomycin, mithramycin, chromomycin, and others) selectively suppress nucleic acid synthesis in tumor and bacteria cells. Kochetkova and colleagues compared the effects of 100 concentrates of new antibiotics in vitro with bacteria cell models (reinforced by data on the mechanism of action) and in laboratory animals with inoculated tumors. Among the substances that selectively suppressed nucleic acid synthesis in bacteria, about 72% also selectively suppressed tumor growth in laboratory animals. In other words, the prognostic value of the bacteria cell model can be improved by approximately 14 times, with the use of data on the mechanism of action of the natural compounds studied.

It is completely clear that the tests performed with tumor cells in vitro can also be improved by additional data on the mechanism of action of inhibitors. In fact, Ivanitskaia et al. (*3*) proposed that the spectrophotometric method of nucleic acid determination be used in primary cultures of suspended tumor cells. This method is simple, reliable, and sensitive, and is recommended for detecting tumor inhibitors. The antitumor antibiotics now used in the clinic actually selectively suppress nucleic acid synthesis in suspended tumor cell cultures.

Some data on this problem are presented in table 1, which shows the effect of certain anticancer antibiotics on the increase in DNA, RNA, and protein in primary suspended cultures of mouse ascites lymphosarcoma strain NK/LI. For comparison, data on the antibacterial antibiotic monomycin (paromomycin), which does not have an antitumor effect, also are presented in the table.

It is clear that dactinomycin and olivomycin selectively suppress RNA synthesis in this system and that bruneomycin and rubomycin have the same action on DNA synthesis. The antibacterial compound monomycin has a completely different effect: It selectively suppresses protein synthesis in this system. On this basis, one can conclude that the introduction of a correction taking into account the mechanism of action of the compound is fully justified in this system of tumor cells in vitro.

TABLE 1.—*Effect of certain antibiotics on synthesis of nucleic acids and protein in suspended tumor cell cultures*[a]

Antibiotics	Concentrations (g/ml) suppressing increase by 50%		
	Protein	DNA	RNA
Actinomycin C (cactinomycin)	0.03	0.03	0.003
Olivomycin	0.01	0.01	0.001
Bruneomycin	0.02	0.001	0.006
Rubomycin	0.002	0.0003	0.001
Monomycin (paromomycin)	125	500	500

[a] *See (3).*

[1] Director, Institute for the Study of New Antibiotics, Moscow, U.S.S.R.
[2] Head, Division of Chemotherapy, Institute for the Study of New Antibiotics.

We can now turn to the second problem in our discussion, which concerns evaluation of the in vitro test system for finding anticancer antibiotics with antimetabolite properties. It should be noted first that in vitro tumor cell models, despite all their advantages, are simply unsuitable for work in this field. The nutrient medium in which tumor cells grow contains a large amount of various metabolites. Most, if not all, antibiotics synthesized by the microorganisms and having antimetabolite properties are neutralized under these conditions and cannot be detected. With the microbe cell systems available, we can use those microorganisms that grow on synthetic media with mineral nitrogen.

Korobkova et al. (*4, 5*) have conducted detailed studies on the problem of screening antibiotics that have antimetabolite properties and are synthesized by actinomycetes. Eight hundred actinomycete cultures were studied in one experiment, and by the use of test bacteria such as *Escherichia coli* and *Flavobacterium devorans*, substances having antimetabolite properties were found. Most of these compounds (about 85%) were neutralized by leucine and had no antitumor effect in tests on animals. However, among the substances (±15%) not neutralized by leucine, some were found which are of considerable interest and which have antitumor activity in vivo. The properties of these inhibitors are now being studied; one of them (antibiotic 1719) is identical to the antitumor antibiotic azotomycin (*6*).

The only eukaryotic cells suitable for the search for new antibiotics having antimetabolite properties are certain yeast cell cultures that require mineral nitrogen and their mitochondrial mutants that have a defect in respiration. It would be tempting to use eukaryotic cells that have a typical nuclear apparatus and mitochondrial system for selection of antibiotic antimetabolites. Their mitochondrial systems are especially interesting, because defects in organization of the mitochondria have been observed in malignant tumor cells. For this purpose, G. F. Gauze and colleagues (*7*) used cultures of two types of yeast, *Candida utilis* strain 766 and *Torulopsis globosa* strain 697, which grow well on synthetic nutrient medium with mineral nitrogen. Mitochondrial mutants were obtained in both types (with small colonies, a defect in respiration, and an increase in glycolysis), which are capable of growing on synthetic nutrient medium. According to data in the literature, irreversible changes in the mitochondrial nucleic acid are characteristic of such mutants.

The production of mitochondrial mutants with small colonies in the yeasts *C. utilis* and *T. globosa* proved to be considerably more difficult than induction of such mutants in the classical species *Saccharomyces cerevisiae*. Nevertheless, with 5 μg/ml trypaflavin (dissolved in nutrient medium) as the mutagen and inoculating yeast cell suspensions 24, 48, and 72 hours after the action of the mutagen at 28° C, several mutant cultures of *Candida* and *Torulopsis* were found. By inoculating the yeast cell suspensions (a Nagai agar diagnostic medium containing a mixture of eosin and trypan blue was used), we noted that the mutant colonies, in distinction from the colonies of the initial cells, had a more intense blue-violet stain.

In the search for new antitumor compounds having antimetabolite properties, the agar block method was used. The actinomycete cultures were inoculated on petri dishes containing the agar medium and organic nitrogen. After 7 days of growth at 28° C, 8-mm (diameter) blocks were cut from the agar and placed on the surface of a petri dish with medium M-9 (mineral nitrogen) and medium No. 2 (organic nitrogen). A suspension of test microbes (cultures of the initial species of yeast and their mitochondrial mutants) was added beforehand to the latter. If growth of the test microbes was suppressed by the agar block on the M-9 synthetic medium but not on the rich nutrient medium No. 2, it could be concluded that an antibiotic having antimetabolite properties was present.

In another investigation, 2,160 actinomycete cultures, freshly removed from various soils, were examined. Data on formation of antibiotic antimetabolites in these cultures, active against the test microorganism of interest to us, are presented in table 2. As is evident, the most interesting results were obtained by the mitochondrial mutant 11-3 of *T. globosa*. This mutant allowed the formation of antibiotic antimetabolites, which could not be revealed by other tests, in 5% of the actinomycete cultures. Further investigations showed that the actinomycete cultures active against the mitochondrial mutant 11-3 of *T. globosa* are inactive toward *E. coli* and its mutant 19-8, which we induced with *N*-methyl-*N'*-nitro-*N*-nitrosoguanadine and which has increased permeability to a number of inhibitors, including dactinomycin. Consequently, *T. globosa* mitochondrial mutant 11-3 is useful for detecting new antimetabolites that are synthesized by microorganisms and are inactive in other tests.

The test systems used in vitro in searching for anticancer antibiotics among the natural compounds are more complicated at the present time than they were 10 years ago. The flow of information from the field of molecular biology on the questions of mechanism of action of antibiotics is leading to greater efficiency in research methods. Further research directed toward making test systems more efficient is urgently needed.

IN VIVO SCREENING

All antitumor compounds used in the clinic suppress growth of one animal tumor or another. This well-established fact is an indisputable basis for the initial selection of new antitumor antibiotics to be used on animals with transplanted tumors. It also is well known that most of the compounds that suppress development of certain animal tumors are ineffective or have little effect in the treatment of human malignant neoplasms. The absence of a clear correlation between animal and human tumors greatly complicates the evaluation of the therapeutic effects of new compounds. Of the blastoma diseases that now occur in animals, we do not know those that would best serve as targets, against which antitumor properties of drugs could be estimated.

No one system is generally recognized for initial evaluation of antitumor compounds. Most proposed methods give good results in the selection of synthetic alkylating compounds and antimetabolites. However, the majority have low sensitivity to the action of natural compounds, particularly of antibiotics. Thus L1210 lymphocytic leukemia, which is widely used in the evaluation of the effectiveness of new antitumor compounds in the United States, does not suppress most antitumor antibi-

TABLE 2.—*Formation of antibiotic antimetabolites in actinomycete cultures studied*[a]

Strain	Growth suppression frequency, %
C. utilis 766 and mutant 12-3	0.004
Mutant 12-3	0.09
T. globosa 697 and mutant 11-3	1.6
Mutant 11-3	5.0

[a] *See* (*7*).

otics, even those in clinical use (olivomycin, dactinomycin, bruneomycin).

Certain specific antiblastoma properties of new antibiotics must be noted. The initial evaluation usually is done with the raw antibiotics, since production of the pure drug involves a laborious process. The raw antibiotics differ sharply from synthetic preparations, inasmuch as the content of active substance frequently does not exceed a few percentage points in the first samples tested. Therefore, detection of the activity of such a compound requires a highly sensitive model. Testing only "pure" antibiotic preparations alone is not advisable, since almost all raw materials are rejected after the first tests. However, treatment of the animals with practically pure synthetic compounds can begin immediately and, for these, excessive sensitivity of a model for the screening is useless and can even be harmful.

Small animals are essential in the screening phase of determining therapeutic effect of new antitumor antibiotics, since efforts to isolate a new and unknown antibiotic frequently result in only a small amount of the preparation, which usually is insufficient for conducting tests on rats and larger animals. Production of a large quantity is hardly justified. Therefore, the most suitable animals for initial evaluation of antitumor antibiotics are mice. Furthermore, a large number of diverse transplanted tumors in these animals have already been described.

Evaluation of the antitumor properties of any raw antibiotic begins with determination of its toxicity. The raw material goes to the chemotherapy section as a dry preparation or an ethanol solution. Usually, three doses of the dry preparation are tested: 10 mg, 3 mg, and 1 mg per mouse. The first dose of the drug in solution is given in not more than 0.1 ml ethanol per mouse, and then two more doses, each one-third of the preceding one. If all the animals die after administration of the three doses, three smaller doses are tested. All doses of the preparations are administered to the mice in the form of solutions or suspensions in 0.5 ml physiologic solution. One-tenth of the lethal dose (LD100) or 0.2 of the mean lethal dose (LD50) is taken as the therapeutic dose. The preparations are administered daily for 6–9 days.

Both toxicity and therapeutic effect are determined by sc administration of the raw material. This is done for the following reasons: First, antibiotic preparations will not always be sterile and may cause bacterial peritonitis in mice. Second, we are convinced that most antitumor preparations are more toxic by administration into the abdominal cavity than sc, since they frequently induce sterile peritonitis because of their high cytotoxicity. For example, the LD50 of rubomycin given sc is over 20 mg/kg; it is about 5 mg/kg by ip administration.

In evaluating the therapeutic properties of new antibiotics suitable for treatment of malignant neoplasms, three transplanted mouse tumors (solid form) were used: lymphosarcoma (strain L10-1), sarcoma 180, and Ehrlich's carcinoma. To obtain the solid form of Ehrlich's carcinoma, 10^6 tumor cells from an ascites tumor in a volume of 0.3 ml are administered sc to mice. Lymphosarcoma L10-1 was inoculated im into the femurs of mice; i.e., 0.15 ml of suspension made from 1 g minced tumor diluted with 19 ml of physiologic solution. Development of sarcoma 180 in mice was induced by sc inoculation of a 2.0×2.0-mm tumor fragment with a trocar. Each dose of the antibiotic under study was tested in 10 mice, and its antitumor effect was evaluated by a comparison of the average weight of the tumors in control and treated mice; the difference in these values was expressed as percent of the average tumor weight in the control.

TABLE 3.—*Results of initial selection of antitumor antibiotics in parallel on three types of tumors: lymphosarcoma L10-1, sarcoma 180, and Ehrlich's carcinoma*[a]

Type of tumor sensitive to new raw antibiotic	Activity confirmed in subsequent tests	Activity not confirmed in subsequent tests
L10-1, sarcoma 180, Ehrlich's carcinoma	12	0
L10-1 + sarcoma 180	9	0
L10-1 + Ehrlich's carcinoma	5	0
Sarcoma 180 + Ehrlich's carcinoma	0	0
L10-1	7	1
Sarcoma 180	0	1
Ehrlich's carcinoma	0	5
Total	33	7

[a] The amount of antibiotic given suppressed tumor growth by 40% or more. *See* (*8*).

A retrospective analysis of the results obtained was performed in 1961 (*8*). In this time, 169 new raw antibiotics were studied. Under the test conditions, suppression of growth of these neoplasms by 30% and more usually corresponded to a statistically significant difference between the average tumor weights in control and treated animals. Therefore, if this difference was 30% or more in the first test, the test was repeated three times and, if favorable results were confirmed, we concluded that antitumor properties were conjecturally present in the antibiotic tested. Analysis of the results revealed that the 3 tumors we used had no uniform sensitivity to the antibiotics that we studied; lymphosarcoma L10-1 was the most sensitive. During the period mentioned, the effect of 133 preparations on growth of lymphosarcoma L10-1 was studied; 45 antibiotics (33.8%) induced suppression of tumor growth by more than 30%. In the first test, suppression of growth of sarcoma 180 and Ehrlich's carcinoma was observed in 25.8 and 25.3%, respectively, of the test compounds (*9*).

The important question is whether these 3 tumors permit selection of different preparations or if they all select one and the same antibiotic. For a solution to this problem, the antibiotics were tested simultaneously on 3 tumors in identical, maximum tolerable doses (MTD) and in the corresponding number of administrations for each model. Forty such tests were arranged; of the 33 antibiotics with reliably established antitumor activity that were found, all suppressed growth of lymphosarcoma. Twelve antibiotics simultaneously retarded growth of the 3 tumors, six suppressed the lymphosarcoma alone, nine simultaneously inhibited development of lymphosarcoma and sarcoma 180, and five simultaneously suppressed growth of lymphosarcoma and Ehrlich's carcinoma. Not one of the antibiotics having significant antitumor activity suppressed only sarcoma 180, only Ehrlich's carcinoma, or the growth of these 2 tumors simultaneously, without suppressing the growth of lymphosarcoma (table 3).

From these tests we concluded that, of the 3 tumors which were used for the selection, strain L10-1 lymphosarcoma was the most sensitive to the effects of various antibiotics. All the drugs having significant antitumor activity suppressed growth of this tumor under our assay conditions, whereas only a few inhibited growth of sarcoma 180 or Ehrlich's carcinoma. Of course, that substances may exist which have a suppressive effect only on sarcoma 180 or Ehrlich's carcinoma cannot be excluded; however, among 169 raw antibiotics studied, such

preparations were not found. In fact, if the initial choice was made only with L10-1 lymphosarcoma, the same result would have been achieved, as was noted in working simultaneously with the 3 tumors. Thus the use of sarcoma 180 and Ehrlich's carcinoma was practically unnecessary. Work with 1 tumor in the initial screening selection of antitumor preparations instead of 3 reduces the extent of the work by two-thirds.

After obtaining these results, lymphosarcoma L10-1 was adopted as the initial screening model. The 2 other tumors and other neoplasms became secondary filters in evaluating the antitumor properties of new antibiotics. Moreover, the goal of finding a model still more sensitive to natural compounds than lymphosarcoma L10-1 was set, and the search continued for several years. At the beginning of the 1960's, mouse lymphadenosis strain NK/LI was obtained from Professor L. Nemetz in Budapest. This ascites form of tumor was included in the initial selection of new antitumor antibiotics; after 2–3 years, this blastoma was still more sensitive to antibiotic substances than was lymphosarcoma L10-1. All antibiotics used at the present time in the clinic were tested on this model, and the results obtained showed that when the MTD were given, all suppressed development of this tumor by at least 80–90%. Almost twice as many antibiotics having antitumor properties were found with lymphadenosis NK/LI as with lymphosarcoma L10-1. Antibiotics suppressing some other transplanted mouse tumor and not retarding growth of lymphadenosis NK/LI have not been successfully detected to date. Therefore, we used lymphadenosis NK/LI in recent years as the first screening model in our selection of antitumor antibiotics. Lymphadenosis NK/LI is transplanted into mice by ip administration of 5×10^6 to 5×10^7 ascites cells that were taken from mice given tumor transplants 7–8 days previously. Treatment begins 24 hours after inoculation and continues for 5–6 days. The therapeutic preparation is always administered sc. We avoided ip injections of the raw antibiotics for the following reasons: 1) to avoid direct contact of high concentrations of the antibiotic with the tumor cells; 2) because the primary raw antibiotics will not always be sterile, they sometimes cause infectious peritonitis; 3) many antitumor antibiotics frequently are considerably more toxic by ip than by sc administration. This difference is especially noticeable in multiple injections. We also recall that antibiotics usually cause a stronger suppression of ascites tumor growth by sc or peroral than by iv administration.

On the day after the last antibiotic dose (usually 7 days after transplantation of the tumor) the mice are killed, the number of ascites tumors in each mouse is determined, and the total number of tumor cells per mouse is calculated. Tumor growth suppression is judged by the average number of tumor cells in treated and control animals; the results are expressed in percent.

When we began to use this screening model, we rejected antibiotics that inhibited growth of lymphadenosis by less than 40%. Preparations suppressing development of lymphadenosis by this percentage or more were tested on other blastoma models: lymphosarcoma L10-1, sarcoma 180, Ehrlich's carcinoma, sarcoma 37, Fisher's lymphadenosis-5178, and others.

Much actual data was gathered in the laboratory by this selection method in more than 3 years. During this period, 713 raw antibiotics were tested for antitumor activity against lymphadenosis NK/LI in approximately 8,000 animals. Of these, 215 were then tested on lymphosarcoma L10-1 (in 2,500 animals), 57 on sarcoma 180 (in 660), 50 on sarcoma 37 (600), 57 on Ehrlich's carcinoma (700), and 46 on Fisher lymphadenosis-5178 (550 animals). A retrospective analysis of the data obtained was of practical interest because we could estimate the effectiveness of the selection system adopted, as well as expose and eliminate certain inherent shortcomings in it. This analysis was done in collaboration with Bazhanov and Kozharinov (*9*).

Of the 713 compounds tested on lymphadenosis NK/LI, 199 (28%) active preparations that inhibited growth of this tumor by 40–100% were selected. In tests on lymphosarcoma L10-1, 78 active preparations (39%) were found among these 199 antibiotics. The largest percentage active against lymphosarcoma L10-1 was found among preparations inhibiting development of lymphadenosis NK/LI by 60% or more when the MTD were given. In subsequent tests of the compounds selected for lymphadenosis NK/LI and active in lymphosarcoma, it was noted that among the strains used (solid form of sarcoma 180, ascites form of sarcoma 37, Ehrlich's carcinoma, and Fisher's lymphadenosis-5178), Fisher's lymphadenosis was the most sensitive to the antibiotics. Of 21 compounds tested on this model, the only inactive compound was the one having the least activity toward lymphadenosis NK/LI. It is possible that NK/LI and Fisher's lymphadenosis are similar cytologically. Sarcoma 180 was the least sensitive model in these tests. Of the 57 preparations tested against sarcoma 180 that were active against lymphadenosis NK/LI and lymphosarcoma L10-1, only 20 (35%) produced a favorable effect.

Since our initial screening model in the selection of new antibiotics having antitumor properties is lymphadenosis NK/LI, interest in evaluating the effectiveness of this system for possible valid choices of its parameters was evoked. For this purpose, a statistical discriminating model was made (*9*), and the probability of first order (rejection of an active compound) and second order (adoption of an inactive compound as active) errors was determined at four different rejection thresholds (table 4).

According to the data presented in table 4, a rejection threshold can be selected in the initial screening of antitumor antibiotics with the ascites form of lymphadenosis NK/LI. This optimum threshold will be the 60% tumor-growth inhibition by sc administration of the compound at the MTD. At this rejection threshold the probability of refusing an active compound is extremely small (less than 0.0005%), and the probability of adopting an inactive compound as an active one is not over 2.5%.

Thus further study on other models of blastomas should be undertaken only for those antibiotics that suppress development of lymphadenosis NK/LI by at least 60% with daily sc administration at the MTD. It is advisable to reject less active compounds, because it is highly unlikely that promising antitumor antibiotics will be found among them.

TABLE 4.—*Effectiveness of system for initial selection of antitumor antibiotics in lymphadenosis NK/LI vs. choice of rejection threshold* [a]

Rejection threshold of tumor growth inhibition, %	Probability of rejection of active compound, %	Probability of adopting inactive compound as active, %
40	< 0.0005	93.8
50	< 0.0005	46.8
60	< 0.0005	2.5
70	7.2200	< 0.0005

[a] *See* (*9*).

Use of the optimum rejection threshold significantly increases the efficiency of screening, since it permits rejection of a considerably greater number of antibiotics in the first tests without subjecting them to additional study. At a rejection threshold of 40% suppression of lymphadenosis NK/LI growth, 199 compounds underwent further study, whereas at a 60%-rejection threshold, only 134 antibiotics would be selected, i.e., 32.7% less. This would exclude needless tests on at least 1,200 animals.

RECOMMENDATIONS

The decision as to which new antitumor antibiotics should be proposed for clinical tests is based on the breadth of their antiblastoma spectrum of action on animal tumors and on their pharmacologic properties. We propose that, primarily, new antitumor antibiotics having the broadest spectrum of action on animal tumors should be studied in the clinic; in the absence of a clear correlation between animal and human tumors, we have more opportunity to find antibiotics suitable for humans when drugs suppress numerous different animal tumors. Of course, we cannot deny that some antibiotics with a narrow spectrum of action on animal tumors may be active against some human tumor. However, the difficulties of clinical testing of such compounds (considering the great diversity of human tumors) should not be forgotten.

We found the following diverse antitumor antibiotics by the selection method described above and modifications of it: dactinomycin, olivomycin (*10*), bruneomycin (*11*), rubomycin (*12*), sibiromycin (*13*), lienomycin (*14*), beromycin (*15*), azotomycin (*16*), carminomycin (*17*), a number of representatives of the echinomycins, etamycin, aureotricin, cinerubin, and iomycin groups, and many others.

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Clinical Test Methods for Antitumor Drugs in the U.S.S.R.

A. M. Garin[1] *and* M. R. Lichinitser[2]

The amount of progress achieved in the treatment of various malignant tumors and leukoses (leukemias) depends on the degree of perfection attained in the clinical testing methods of antitumor drugs. In the U.S.S.R., testing of these drugs is guided by experience and the recommendations of the Committee on Controlled Clinical Testing, International Anticancer Union. We report here some of our ideas and approaches developed in our studies of new antitumor drugs.

Four phases of study of a new drug make it possible to evaluate its effects on various tumors and on the body, the selectivity of antitumor activity, and the fundamental innovation or advantages of this drug over others, which ultimately determines the place of a new compound in cancer treatment.

PHASE I

In 1966, Professor Astrakhan (*1*) proposed that the main task of phase I be the determination of the maximum tolerable dose (MTD) in man in a daily, 30- to 35-day course. This course dose should cause reversible side effects in 50% of the patients.

Despite various approaches to phase I in recent years in different countries, the principle as stated is the most valid. Use of a prolonged course decreases the risk of delayed side reactions long enough for scientists to determine its potential effects, which, during phase II and administration of an adequate course dose, can then be evaluated with the selectivity of its action. This method is particularly important for test compounds given orally because of the different patterns of intestinal absorption in individuals. Also, short (3- to 5- or 5- to 10-day) and interrupted treatment regimens are necessary.

The interrupted treatment method has been used in the U.S.S.R. for many years. It is based on the work of G. L. Zhdanov who, in studying the effect of embichin on normal and tumor tissues (1955–60), determined that the regeneration time after the damaging action of cytostatic substances was not uniform in all tissues. Proliferation of the intestinal epithelial and bone marrow cells was restored most quickly of all, the lymphoid organs somewhat later; suppression of tumor growth continued longer than any other action. On the basis of this, a regimen of administration of embichin once every 7 days was proposed. At the present time, this regimen is advised for dopan, sarcolysin, and some other drugs.

Experience is being accumulated on the benefits of a short (5-day) course of treatment, which is used most frequently in other countries for phases I and II. The main shortcomings of this regimen are the difficulties involved in a significant number of patients receiving the course MTD and in determining the proper dose measurement for previously treated patients, etc. Short regimens occupy an important position in the treatment of patients with certain forms of malignant neoplasms, but they cannot be acknowledged as being preferred in initial clinical tests (phases I and II).

The phase I method has now been finalized and is directed toward producing several regimens for use of a new compound (prolonged or 20–30 days, short or 5-day course, interrupted or 1–2 times per week). It is assumed that 1) accumulation of a toxic effect of a drug is the greatest in a prolonged course; 2) detailed characteristics of toxicity manifestations can be obtained; and 3) a treatment regimen can be curtailed with less risk to the patient. On the other hand, a change from a 5-day regimen to a 20- to 30-day one is a more difficult matter. Information on the side effects of the drugs in a 30- to 35-day course of treatment was used to develop that approach.

Preclinical investigation in laboratory animals (mice, rats, cats, and dogs) gives an idea of the LD10 in one-time and multiple administrations, whereas the LD50 characterizes the effect of the compound on hematopoiesis and functioning of the liver, kidneys, and heart, etc. The dynamics of distribution of the drugs in various media and tissues, as well as the antitumor therapeutic index, are successfully ascertained.

To obtain a 20- to 30-day regimen for man, the course MTD of mice or rats in a 15-day administration per milligram/square meter of body surface is used. The one-time dose, calculated as 1/20 of the course dose, which is conditionally tolerated by man, is administered daily for 20 days. Three principles are used in sequence for increasing the course dose: prolongation of treatment from 20 to 30 days, maintaining the size of the one-time dose, and increasing the one-time dose by a modified Fibonacci scheme with continual fluctuations in duration of treatment between 20 and 30 days (table 1). Each stage of the increase in course dose is evaluated in 2 patients, and the interval between times of inclusion of patients in the investigation is 1–2 weeks. The work is considered completed when a course dose causes side effects. This dose should be given to 4–6 patients. Such a test system permits a 20- to 30-day treatment regimen to be obtained in 2–4 steps in 8–12 patients; the compound can then progress to phase II study.

However, phase I should be continued. Development of 5- and 10-day or interrupted regimens is a difficult and dangerous matter. The differences in cumulative effect of various antitumor compounds and the unusual ratio of the course doses given in long and short treatment periods should be kept in mind. Animal experiments provide examples of unusual accumulation capacities. For example, dizaet in mice has a MTD7 which is 170 times less than the MTD1 (A. B. Syrkin and colleagues).

We are now using two approaches in the development of 5-day courses. In a study of carminomycin, the first patients received half the course MTD for dogs (15 mg/m^2) administered iv at the rate of 1.5 mg/m^2 daily for 5 days. Two increases in the one-time dose were used (twice and 3.3 times) to reach moderate toxicity. We wish to emphasize that preliminary

[1] Head, All Union Cancer Chemotherapy Center, Cancer Research Center, Moscow, U.S.S.R.
[2] Department of Chemotherapy, Cancer Research Center.

Phase I	Phase II	Phase III
Determination of MTD in 20–30-day treatment (regimen 1)	Study of effectiveness in signal tumors (regimen 1)	Comparison with known drugs
Determination of MTD in 5–10-day treatment (regimens 2, 3)	With favorable result of phase A ↓	Study of cross-resistance
Clinico-pharmacologic evaluation of effect of drug on body	Study of effectiveness in signal tumors (regimens 2, 3)	Determination of effectiveness of drug combinations
	Study of effectiveness in supplementary spectrum of tumors	
	Detailed clinico-pharmacologic evaluation of effect of drug on body	
Decision on advisability of practical use →		Phase IV
		Inclusion of new compound in general plan of treatment of oncologic patients

TEXT-FIGURE 1.—Stages of clinical testing of new antitumor drugs.

TABLE 1.—*Method of compiling a prolonged regimen for 2 patients*

One-time dose, mg/m²[a]	Percent increase in dose	Duration of treatment with daily administration, days
p	—	20
p	—	30
2 p	100	20
2 p	100	30
3.3 p	67	20
3.3 p	67	30
5 p	50	20
5 p	50	30
7 p	40	20
7 p	40	30
9 p	30	20
9 p	30	30
12 p	30	20
12 p	30	30

[a] p = one time, 2 p = twice, etc.

clinical study of the side effects in a prolonged course of carminomycin treatment in man established conditions for a more rapid and a safer study of the short regimen.

The other approach was used in testing the compound imidaphen, which is distinguished by a low capacity for cumulative effect on hematopoiesis. The treatment period was reduced sequentially by two and four times, with a corresponding increase in the one-time dose of 2 and 3.3 times. Leukopenia and thrombocytopenia delayed by 2–3 weeks was observed, and the course doses approximated each other in both regimens (800–1,000 mg/m²). Despite the success in the example presented above, this route is considered dangerous, and it is not used in new investigations.

Great hope is placed on the development of the principle of dynamics of toxicity accumulation in laboratory animals proposed by M. R. Lichinitser and A. B. Syrkin in 1973. The MTD and LD50 in 1-, 3-, 5-, 10-, 15-, 20-, and 30-day administrations is determined in the preclinical phase for each new compound. The information obtained makes it possible to show graphically the MTD variations in different periods of administration of the drug, to compare a new drug with compounds close to it and, after accumulation of clinical experience, to compare the dose in animals and man as functions of treatment periods. The results of this work should serve as experimentally valid principles for change from a long regimen to a short one and vice versa. Serious attention is given to tolerability of a one-time drug dose in phase I. A small one-time dose of some peptides of sarcolysin given perorally caused sharp nausea and vomiting. Therefore, high one-time doses were recommended, and short regimens could not be used. Asaphan caused nausea and vomiting in 71 of 184 patients (40%), asaley in 149 of 282 (53%), and phenamet in 106 of 214 patients (50%). With the examples of phenamet and asaley, the manifestations of direct toxicity do not decrease with administration of a daily dose two, three, and four times the amount given initially. However, a 50% reduction in the one-time dose partially decreases the side effects. An increase in the dose of sarcolysin peptides that produces nausea constantly leads to more pronounced side effects. Direct side reactions usually increase toward the end of a 20- to 30-day course.

The matter is somewhat different with a patient's ability to tolerate a one-time dose of prospidine or methylnitrosourea: Direct side effects of prospidine (dizziness, feeling of intoxication) and methylnitrosourea (vomiting) can be decreased or stopped by prolongation of the treatment. The dose of olivomycin cannot be raised above 10–15 mg, even at the cost of increasing the intervals between administrations.

For phase I clinical testing, a one-time dose is selected that does not cause any direct side reactions in a prolonged course, but in the short regimen, measures are taken to decrease the side effects with antiemetics and other agents so that an adequate course dose can be given. Phase I is conducted according to a specific protocol in which special attention is given to selection of patients and the characteristics of side effects.

Patients selected for treatment with a new drug for which the toxicity has not been determined are those with 1) untreated neoplasms, 2) no prospect of effective therapy by other agents, 3) a poor 6-month prognosis, and 4) no treatment with cytostatic substances in the previous 2 months. The side effects are evaluated by the standard set of laboratory tests, and the most toxic, revealed in preclinical study and during clinical testing, are subjected to detailed study, e.g., bone marrow hematopoiesis in assays of phosphamide, benzo-TEPA, and other ethyleneimides, liver function during a prolonged dichlorodiaminoplatinum course, etc. Evaluation of the side effects continues for 3 weeks after completion of a 20- to 30-day course of treatment and for 4–6 weeks after the short course.

Standardization of the side reactions caused by antitumor drugs on hematopoiesis, the digestive tract, skin, etc., is important at all stages of clinical testing and, in particular, in phase I. A classification of those most frequently seen that require interruption of treatment in phase I is presented in table 2.

TABLE 2.—*Most frequent side effects requiring interruption of treatment in phase I*

Degree	Side effect		
	Leukopenia: WBC/mm³	Thrombocytopenia: platelets, mm³	Diarrhea, times/day
Moderate	4,000–3,001	150,000–101,000	3–6
Considerable	3,000–2,001	100,000– 70,000	
Sharp	2,000–1,001	70,000– 51,000	8
Threatening	1,000 and below	50,000 and below	

Phase I establishes the course dose of a drug that causes severe side reactions. Refinement of the safety of a dose and its adequacy (tending toward side effects in 50% of the patients) is continued at the start of phase II testing. Sometimes, unusual side effects indicate the necessity of returning the compound from phase I clinical testing to further preclinical study.

Aziprin, 200–400 mg iv daily or every other day for 20–30 days, caused neuropsychic disorders in 11 of 19 patients with various malignant tumors. Headache, dizziness, insomnia, agitation, nystagmus, trembling, and hyperkinesis of the muscles were noted in all 11 patients. These symptoms, which developed gradually in the middle of the course and increased with prolonged administration of the drug, were sharply expressed in 4 patients, and visual and auditory hallucinations (a unique pattern of a toxic psychosis) appeared. The neuropsychic disorders, directly related to dose and intensity of treatment, developed with a dose of 5,200–7,200 mg (92–98 mg/kg) in 27–30 days.

In connection with this, a study was conducted of the effect of aziprin on the electrical activity of the rat brain (I. P. Anokhina). After ip administration at doses of 50–350 mg aziprin/kg, synchronized rhythms of 6 or 9–10 oscillations per second appeared on the EEG. Synchronization was brief initially and then more prolonged, appearing first in the subcortical sections and then in the cerebral cortex. If 3 mg haloperidol/kg is administered sc *after* aziprin, the synchronization is strengthened. However, aziprin did not affect the EEG with *prior* administration of haloperidol. The positive effect of haloperidol by administration before aziprin was tested in patients and, in this case, development of neuropsychic disorders was successfully decreased. This creates more favorable conditions for phase II clinical testing of aziprin.

In *Methodical Indications for Initial Clinical Study of New Antitumor Drugs,* compiled in 1966 by V. I. Astrakhan, the initial daily dose for drugs of the chloroethylamine and ethyleneimine groups is 1/30 MTD (in milligrams/kilogram) for 15 days for mice or rats. This one-time dose is given to patients for 30–35 days. Data analyses show that this more cautious approach is important in testing ethyleneimines, which have delayed effects on hematopoiesis, even in a 20- to 30-day course.

In the study of compounds closely related in chemical structure or mechanism of action, it was impossible to formulate a regimen based on a comparison of the MTD and LD50 of the new drug with those of a known one. Thus the MTD of phosphamide in mice is three times greater than that of thio-TEPA, but the doses were equal in the clinic setting.

Inclusion of development of the tolerable dose in regional administration in the phase I method has a special place. This is particularly necessary for drugs undergoing rapid metabolic conversion and for highly toxic compounds. The method of conducting these antitumor drug assays with dogs was developed by S. A. Gasparian, L. V. Moroz, and others (unpublished observations). Subsequent clinical tests in man revealed the high effectiveness of thio-TEPA and sarcolysin in cancer of the larynx, oral cavity, tongue, and certain other tumors.

Thus fractional administration of thio-TEPA and sarcolysin in cancer of the larynx, through the upper thyroid artery, caused complete regression in 4 of 14 and 3 of 11 patients, respectively, and considerable reduction in the tumors (>50%) in 9 and 3 patients, respectively (Titievskaia V V: Unpublished observations). That iv administration of thio-TEPA and sarcolysin to patients with such types of tumors is usually ineffective is well known.

It must be kept in mind that development of the regional infusion method in phase I is linked with administration of a high concentration of the compound and better use of its specific action (e.g., the high activity of sarcolysin under conditions of regional alkalosis or an increase in effectiveness by continuous administration.

PHASE II

The main objective of phase II clinical testing is to determine the effectiveness of a new drug on various malignant tumors. In the preliminary stages of the work, refinement of the tolerance to the course dose continues in patients previously untreated or those receiving intensive drug and radiation therapy; efficient one-time and course doses for repeated treatment are developed.

If a drug is effective in a prolonged course, it is used in other (short or interrupted) regimens. Detailed clinical and pharmacologic analyses of the new drug are continued. Simultaneously, efficient regimens in combination with other cytostatics or with radiation, which are proposed for study in phase III, are prepared.

Before work is started, a phase II clinical study protocol is compiled, in which the results of phase I: first information on side effects, singularities of the new drug, the plan of study, treatment regimen, plan for examination of patients, criteria for evaluation of the therapeutic effect, recommendations of one-time and course doses in treated and untreated patients, etc., are reported.

Each new compound should be studied in cancer of the lungs, breast, stomach, and ovaries, as well as reticulosarcoma,

lymphogranulomatosis, melanoma, and lymphoblastic and myeloblastic leukemias. Among the tumors named, there are slow- and fast-growing ones, different degrees of tissue differentiation, some with singularities of metastasis and clinical course, and those with different histogenesis.

For the initial clinical test, it is important to have convincing objective criteria of the therapeutic effect before giving treatment. Because of the difficulties of evaluating the results, we do not include cancer of the pancreas or colon, or malignant tumors of the brain among the signal tumors. There should be at least 14 patients with each signal tumor under conditions of "absence of an effect" to have assurance that the compound, if it is effective in a given tumor, will be so in at least 20% of the cases. Recently, we attempted to increase the number of subjects with lung cancer who are included in the investigation of a new drug and consider it necessary to have at least 14 patients for each study of small-cell and flat-cell cancers.

Phase II is conducted only on patients with untreated tumors, who do not have a chance of being cured by known agents. In the first stages, it is advisable to treat those who have never received cytostatics or undergone intensive chemotherapy for more than the previous 2 months. Patients who received intensive drug therapy should not be included. The course dose is refined in the long regimen to produce reversible side effects in approximately 50% of the patients. One-time and course doses and rational intervals for repeated courses of treatment are determined simultaneously.

Sometimes the course MTD of some drugs must be reduced in repeated therapy. For example, the dose of fluorobenzo-TEPA, which causes leukopenia and thrombocytopenia, had to be reduced by one-fourth.

Second courses must be administered when no benefit has been derived from the first; a preliminary evaluation of a test drug is made after the first regimen and a final evaluation after two courses. If considerable therapeutic effect is demonstrated, three, four, or more courses are given and, with a partial non-incremental effect after two, the new drug can be cancelled or supplemented with other agents.

After the effectiveness of a long course is determined, study of the short or interrupted systems of treatment begins (with 10–15 patients having each tumor); this approach is justified based on past experience. For example, for some vague reason, only the short regimens of methotrexate and rubomycin and only the long regimens of sarcolysin, etc., were used for many years. At the present time, the regimens followed are based on tradition rather than on sound clinical experience.

Criteria for direct evaluation of an objective therapeutic effect is a serious problem in phase II study. The recommendations reported in *Methodical Indications for Initial Clinical Study of New Antitumor Compounds* are used for measurable tumors:

Degree of improvement	Status of tumor and metastases	Score
Definite	Disappeared or decreased by at least 50% in at least 1 month	3
Significant	Decreased by 25–50% in a period of at least a month	2
Negligible or brief	Decreased by less than 20% or the decrease was maintained for less than a month	1
None	—	0

However, the possibility of refining the general and particular criteria for evaluation of therapeutic effect in various tumors and leukoses is now being discussed and studied.

Thus in lung cancer, an expressed improvement would be demonstrated by a decrease in tumors and an increase in the lumina of the bronchi, complete regression by disappearance of any neoplastic development (previously detected by X-ray and which should be ascertained by broncoscopic examination), and negligible therapeutic effect by decreased inflammation and an improvement in ventilation but the primary symptoms remain.

If necessary, at the end of phase II clinical tests, a new drug is combined with a known one, and the combination (given to 5–7 patients) is then refined for detailed study in phase III. In making up such combinations, the course dose of the new drug should not be less than 50% of the normal total dose.

Evaluation of the results of phase II study of a new antitumor drug is conducted according to the following criteria: 1) frequency of direct objective therapeutic effect; 2) selectivity of antitumor effect; and 3) length of remission.

A drug with antitumor activity on two or more signal tumors undergoes study in an additional spectrum of human tumors (sarcoma of the soft tissues, brain tumor, cancers of the colon, tongue, oral cavity, and esophagus, and neuroblastoma and nephroblastoma of children).

Ascertaining the frequent appearance of a direct, objective therapeutic effect in phase II allows us to consider the importance of the new drug in treatment of tumors that are resistant or have little sensitivity to known agents; it is the main factor in stopping tests of planning phase III. An additional criterion is evaluation of the duration of remission, which, in effective therapy, is measured from the beginning of treatment to re-emergence of symptoms.

The second task is evaluation of the selectivity of the antitumor effect of the drug. In the long regimen, the minimum therapeutic dose (TED) or that amount of the drug that causes 50% regression in tumor size is determined. The ratio of the TED to MTD is the selectivity coefficient of the antitumor effect. It is proposed that this coefficient may be useful in the preparation of drug combinations.

How to study hormone preparations remains unclear. Considering the importance of suppression of the follicle-stimulating hormone (FSH) of the hypophysis in the therapeutic action of androgens, it is advisable to determine the degree of suppression of FSH in administering high one-time doses of testosterone for 1 month. This establishes a basis for comparative approbation of new hormone preparations. The administration of the one-time dose during prolonged hormone therapy for 1 and 3 months, study of the biologic effect, side reactions, and antitumor activity in man is combined in phase I and II clinical testing.

These investigations require careful clinico-pharmacologic analysis. A study of the biologic and antitumor effects of 17α-hydroxyprogesterone capronate (25% p-p) in menopausal patients suffering from breast cancer was concluded recently. The compound was given im at a dose of 1.5–2.0 g daily for 1.5 months, with subsequent reduction to 1 g every other day. A considerable increase in 17-ketocorticosteroids in the urine was found; some patients had an increase in FSH and a considerable reduction in estrogens and pregnanediol. These changes disappeared when the dose was reduced to that normally used (N. I. Lazarev and colleagues: Unpublished observations). High doses of 17α-hydroxyprogesterone capronate caused a sizable increase in fibrinogen content, fibrinolytic activity, and in antifibrinolysin level. An antitumor effect was produced in 5 of 14 patients, with the duration of remission from 4 to 10 months.

PHASE III

The objective of phase III clinical testing is extensive study of new compounds. A comparative survey is made of drugs that are similar in chemical structure, most effective, and that have cross-resistance with preparations having another mechanism of action. The usefulness of the new drug in combined therapy and the characterization of the side effects are determined.

All studies in phase III were accomplished by the comparison method, with random selection of patients in the group. We now use the "converter" method, in which each patient's treatment regimen is specified. The second rule in conducting phase III consists of precise characterization of the group of patients before treatment, as a function of age, tumor size, morphology, prognosis, and other factors ("stratification"). These data can be studied in planning the investigation ("preliminary" stratification, e.g., a comparison of two drugs given to patients with small-cell lung cancer), which is always necessary in the analysis of the final results ("retrospective" stratification).

Comparison of a new substance with a known one permits a more distinct evaluation of the differences in antitumor activity. Thus when patients with ovarian cancer were given asaley (sarcolysin peptide), 9 of 36 (25%) received considerable benefit.

An analysis of data on sarcolysin in the literature showed that it was effective in 30–70% of the patients. A comparison of asaley and sarcolysin in equally toxic regimens was undertaken by the double-blind method, in which both drugs were coded for the attending physician and patient; asaley and sarcolysin had nearly the same effectiveness (29 and 32%, respectively). The double-blind method is principally used for comparative studies of antitumor drugs for which the criteria for evaluation of their therapeutic effect (cancer of the stomach, pancreas, and others) have not been clearly defined.

Evaluation of side effects is important for characterization of the drug. For example, it was found that novembichin, dopan, and sarcolysin (with respect to the leukopenic period and its phases of decreased and partial restoration) did not differ from one another, and endoxan has a shorter phase of partial restoration of leukocytes in the blood (*2*).

The negligible effect asalin has on hematopoiesis permitted its use in initial leukopenia (*7*). Similar information was obtained for phenamet. On the other hand, the MTD of bruneomycin caused considerable suppression of hematopoiesis for a prolonged time.

Development of drugs that are effective though resistant to other agents remains the most urgent task of researchers in cancer and leukosis chemotherapy. Thus sequential use of methylnitrosourea after cyclophosphan and vice versa revealed the absence of cross-resistance of these drugs in lung cancer (*9*). Bruneomycin causes no more remission in lymphogranulomatosis than does dopan or vinblastine, but with no cross-resistance, it proved to be highly effective in difficult, resistant cases of a disease involving the retroperitoneal lymph nodes, liver, and spleen (*3, 10*).

It was ascertained in testing phenamet that remission during the IIIB–IV stage of lymphogranulomatosis was achieved in 77% of the patients, some of whom experienced no suppression of hematopoiesis after repeated chemotherapy and intensive radiation treatment. Apparently, it is correct to study phenamet + vincristine + natulan + prednisolone, in a comparative examination with the frequently used combination of embichin + vincristine + natulan + prednisolone.

The principal basis for the study of any new drug in combination during phase III is the discovery of overcoming drug resistance to known preparations. Nontoxic treatment regimens are subject to detailed study in phase III investigations, with attention given to the advantages of certain drugs in their selectivity of antitumor effect. Analysis of clinical experience in chemotherapy of Wilms' tumor and sympathoblastoma in children shows that during a 20- to 30-day course of treatment, maximum effect is reached when 50–75% of the approximate course dose is given, which is long before side effects appear. This is the guideline for repeated, short courses of treatment to patients who demonstrate beneficial effects and for rejecting continuation when therapy is unsuccessful.

Three phases of clinical testing of new antitumor drugs serve as criteria for release of the compounds to practicing physicians. They are: 1) effectiveness of at least 20% in malignant neoplasms not responding to treatment with known drugs; 2) improved effectiveness or less toxicity (with an equal antitumor effect) over drugs similar in chemical structure; and 3) high antitumor activity of a new compound of different chemical structure (even at equal effectiveness with known drugs). The decision to use a new drug in the practice of medicine should not cancel further study.

PHASE IV

We consider it essential to emphasize phase IV, in which the most efficient use of a compound is determined and the up-to-date possibilities and deficiencies in the general plan of treatment are clearly defined, with allowances for detailed characterization of the disease attained by stratification. This opens the way to refinement of the most efficient individual treatment of patients with various neoplasms.

Phase IV should answer the questions on 1) the sequence in which drugs should be used, 2) the place of a new compound among them, 3) whether a long course of treatment to maintain the remission is advisable, and 4) whether a drug is useful for the prevention of metastases after operations or in the early stages of the disease, etc. Although the method of answering the first two questions is based primarily on the principles used in phase III of the investigation, the approaches to postoperative therapy are not well developed and need improvement.

The first principle for additional therapy after an operation is based on the many-sided clinical characterization of the disease before and after the operation for individual patients and for the entire group. It is advisable to distinguish categories of patients with good and poor prognoses. Patients with the most typical course of the process for a given disease belong in a separate category.

The decision regarding a patient's "oncologic operability" requires preoperative measures such as scanning and angiography of the liver, cytologic examination of the lymph nodes and bone marrow, etc. After the operation, we look for tumor cells in the blood and study tumors producing hormones (gonadotrophins in metastases of chorioepithelioma of the uterus and testicles; ACTH-like substances; antidiuretic parathyroid and other hormones in cancer of the lungs, kidney, and ovaries; catecholamines in sympathoblastoma, etc.). Dynamics of embryo-specific protein content in cancer of the liver, teratoblastoma, and cancer of the colon, evaluation of the dynamics of immunodepression by skin tests with dinitrochlorobenzene (DNCB) or with tuberculin, and the dynamics of cytotoxic

activity of the lymphocytes, etc., are also reviewed postoperatively.

The second principle to be observed is the necessity for comparative studies. After radical operations, patients are divided into groups so effectiveness of supplementary therapy can be determined. Divisions into a common group or into a specifically formed one, e.g., those with poor prognosis, or with anergia toward DNCB, can be used. However, such grouping by clinical characteristics can be made during analysis of the results. The best method of grouping the patients is by the converter, with specification of one of the treatment regimens. Selection of the method of supplementary cytostatic therapy after surgery depends on the scientific task (primary search for prophylactic chemotherapy, effort to improve modern treatment, or use of standard therapy). Each objective needs refinement or a solution for different malignant tumors.

In conducting the primary search for prophylactic chemotherapy, a comparison should be made (without fail) of untreated groups of patients who enter the clinic at the same time. Such a control is necessary not only for confirmation of the favorable results but also for timely exposure of unsuccessful treatment, which, unfortunately, is real and has been confirmed. Brunner (1971, unpublished observations) established that development of metastases increased in cancer of the lungs as a result of prolonged use of endoxan after a radical operation.

The principle of treatment in the observation group, as formulated above, depends on whether effective therapy is available for a given tumor (to give a better method) or does not exist (placebo, observation). A similar approach can be used in chorioepithelioma of the uterus, sympathoblastoma of children, late surgical stages of cancers of the breast, lungs, tongue, and ovaries.

Preoperative chemotherapy is safe and should be administered in most cases; postoperative chemotherapy appears desirable particularly when the patient has a poor prognosis. Preoperative treatment can be tested with drugs having even 20% activity, on the theory that the effects of a given drug in tumor metastases is of practical value (i.e., the tumor decreases more than 50% in size). In conducting a preoperative test, several circumstances should be taken into account:

1) The treatment should be conducted after a precise diagnosis.
2) It is inadvisable to use those drugs that cannot be confidently evaluated for therapeutic effect.
3) Preparations with an undetermined antitumor activity must not be administered.
4) Drugs or doses of drugs that complicate the subsequent operation must not be used (e.g., mithramycin causes hypocoagulation; 5-fluorouracil, reduction in regeneration; and other drugs (in specific doses), leukopenia and more frequent occurrence of infectious complications, etc.).
5) In preliminary experimental and clinical investigations, it is desirable to evaluate the immunodepressive effect of a compound (highly unlikely in a short course), relationships to anesthetics, and other pharmacologic effects.
6) It is advisable to use a short course of treatment (not over 2–5 weeks) with administration of 50–75% of the approximate course dose.

The time element for starting postoperative therapy is of great importance. Usually, postoperative chemotherapy is started 1 month after surgery. By this time, the possibility of postoperative complications has disappeared, side effects of anesthesia are eliminated, and the general state of the patient's health has been restored to normal. Because of the frequent occurrence of metastases postoperatively, an interval of only 1 month is essential. Maximum therapeutic effect of drugs can best be achieved when few tumor cells are present (immediate postoperative period).

After a tumor is removed, conditions can arise that prevent formation and growth of metastases. In hypernephroid cancer, removal of the tumor sometimes leads to resorption of even obvious metastases. In a series of studies, Hellström (1972) showed that removal of a tumor caused the disappearance of a factor that had previously blocked the cytotoxic action on the tumor by the patient's lymphocytes; i.e., the destructive effects on the tumor cells were immunologic.

In connection with what has been stated above, and with experimental information on the ineffectiveness of many drugs with respect to "latent" tumor cells, we are interested in the study of "delayed" chemotherapy after an operation. The time to start delayed chemotherapy is defined as three-fourths of the period from the operation to that time at which metastases usually appear. A group of patients in whom it appears possible to make an early diagnosis of metastatic growth (renewal of hormone secretion by the tumor, appearance of serum-blocking factor, embryo-specific protein, etc.) can serve as the controls. The use of drugs to cause manifestation of these symptoms before metastases appear can be called "early treatment." The effectiveness of the so-called "early prophylaxis," "delayed prophylaxis," and "early treatment" must be compared.

The dose regimens for drugs can be of critical importance. In administering a compound during an operation, it is desirable to select a dose that does not cause direct or delayed side effects. During the first month after surgery, the course dose can be reduced 50–75%, allowing for an increase in hematologic toxicity of thio-TEPA, cyclophosphan, 5-FU, and other drugs. The drug dose can also differ later during recovery; this can best be determined on an individual investigation plan. The duration of postoperative therapy is especially important to success. The negligible achievements of short-term prophylaxis can be partially explained by the incorrect assumption of rapid death of the cancer-cell population.

Well-known achievements in treatment of acute lymphoblastic leukosis and stages III–IV lymphogranulomatosis by intensive, prolonged chemotherapy proved the imperfections in previous chemotherapeutic concepts in oncology. The change to prolonged prophylactic use of dactinomycin (75 μg/kg every 3 months for a period of 2 years) actually increased the number of children cured of Wilms' tumor. The choice of the treatment period in malignant tumors among adults does not now have a solid scientific basis. Doubling the time to the average approximate time metastases appear can be used as a working hypothesis. The treatment can be interrupted or continuous. Short, interrupted courses have a number of advantages because they 1) are convenient for bedridden and ambulatory patients, 2) should not cause toxicosis, and 3) have a weak immunodepressive effect.

An equally complicated and urgent problem of phase IV tests of new antitumor drugs is their accessibility for use in the early stages of treatment of various neoplasms. From the research point of view, it is desirable to experiment if one considers the number of favorable factors for the action of the compound (relatively low number of tumor cells, negligible effect of the tumor on the body, preservation of immune reactions, etc.). From the medical point of view, there is a danger that the drug may harm a patient who might be cured by a timely operation or radiotherapy.

A rational method of testing new drugs in early forms of malignant tumors with the medical and research approaches has not been developed. It is advisable to segregate a group of patients who have a poor prognosis despite early diagnosis and to develop a system of stepwise clinical administration of drugs at various stages of the disease. Uncontrolled chemotherapy for early forms of malignant tumors is not recommended.

The principal objectives of future investigations in clinical testing of antitumor drugs are the following:

1) To develop a method of conducting phase I testing of compounds of differing chemical structures and mechanisms of action among the classes of antitumor compounds;
2) To improve clinical laboratory methods characterizing distribution and metabolism of the compound and its biologic effect at the body and cell levels;
3) To continue various studies directed toward refining the clinical and metabolic manifestations of the disease in each patient for the purpose of more distinct grouping during clinical tests of drugs;
4) To develop comparative studies to clearly define the criteria of impossibility or inadvisability of such studies;
5) To develop unified criteria for evaluation of the results of chemotherapy of various tumors;
6) To define a method of selection of drugs for postoperative chemotherapy;
7) To define a method of testing combinations of drugs and radiation therapy;
8) To find a method for approval of new compounds, based on original ideas (interferon inducers, other substances);
9) To define a method of testing combinations of modern cytostatics with immunologic reactions;
10) To work out stages and principles of use of new effective drugs in patients with various stages and prognoses of a disease;
11) To develop international cooperation in compiling unified principles of clinical testing of antitumor drugs.

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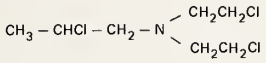
Soviet Antitumor Preparations

V. I. Borisov[1]

A brief characterization of Soviet antitumor compounds widely used in clinical practice is given in this paper.

Novembichin

Novembichin was first offered in 1950 (*22*). Chemically, it is 2-chloropropyldi(2-chloroethyl)amine hydrochloride and has the following formula:



Although novembichin is similar to embichin in pharmacologic properties, a detailed study demonstrated that its action on bone marrow is weaker than that of embichin.

Novembichin, used mainly for treatment of lymphogranulomatoses, produced a direct, favorable effect, but at the present time, it is rarely prescribed by oncologists, due to its high toxicity for hematopoiesis. The compound is produced in 10-mg ampuls; the one-time iv dose is 8–10 mg three times/week and the course dose is 80–120 mg. A summary of its effectiveness against some tumors is presented in table 1.

TABLE 1.—*Antitumor effectiveness of novembichin*

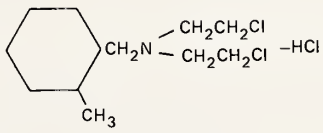
Tumor type	Number of patients	Objective effect	No effect
Chronic myeloleukosis	12	7	5
Chronic lympholeukosis	7	7	—
Lymphogranulomatosis	65	55	10
Reticulosarcomatosis	10	1	9
Lung cancer	8	—	8
Total	102	70	32

Novembitol

Novembitol was synthesized by Rapp and Kornev at the Kiev Institute of Pharmacology and Toxicology. The compound is ortho-xylyl-di-(β-chloroethyl)amino hydrochloride (*30*).

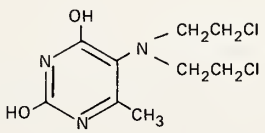
Experimentally, it suppresses growth of various tumors (Geren's carcinoma, Ehrlich's carcinoma, Crocker's sarcoma, rhabdomyosarcoma). Compared with other compounds, novembitol affects bone marrow hematopoiesis slightly. The mean lethal dose (LD50) for mice is 150 mg/kg.

In clinical use, it was active in lymphogranulomatosis, but now is rarely used for tumor therapy since its effectiveness is slight. The one-time dose is 45 mg iv every other day, whereas in a course of treatment, 700–900 mg of the compound could be given.



Dopan

Dopan is 4-methyl-5-[di(β-chloroethyl)amino]uracil.



Dopan was synthesized in 1954 (*23*), as a result of studies based on the general idea that antitumor compounds could be formed by connecting cytotoxic groups to metabolites. Dopan is similar to embichin in its pharmacologic activity. Upon administration of toxic doses of this drug to animals, aplasia of the bone marrow develops, and necrotic changes in the mucosa of the gastrointestinal tract are found.

The degree of suppression of hematopoiesis depends on the dose; granulocytopoiesis is affected first, followed by the production of lymphocytes, erythrocytes, and blood platelets. Other side effects include nausea and vomiting. The antitumor spectrum of dopan differs considerably from that of embichin in experiments. Dopan cures sarcoma 45 and inhibits growth of sarcoma M-1 and Geren's carcinoma; mouse tumors are less sensitive to dopan.

When first given to patients in 1955, dopan had a pronounced antitumor effect in lymphogranulomatosis and chronic myeloblastic leukosis, with splenomegaly and chronic lympholeukosis (*1, 6, 24*). Its effectiveness in these trials is presented in table 2.

The compound is given clinically in a one-time dose of 8–10 mg, once every 5 days; 60–80 mg is administered in a course of treatment.

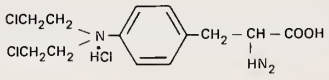
[1] Cancer Research Center, Moscow, U.S.S.R.

TABLE 2.—*Antitumor effectiveness of dopan*

Tumor type	Number of patients	Objective effect	No effect
Lymphogranulomatosis	160	131	29
Chronic myeloblastic leukosis	7	6	1
Reticulosarcomatosis	17	2	15
Reticulosis	8	2	6
Lung cancer	5	—	5
Breast cancer	3	—	3
Chorioepithelioma of the testes	4	—	4
Kaposhi angiosarcoma, cancer of the bile ducts, ectopic chorioepithelioma of the testes	3	—	3
Total	207	141	66

Sarcolysin

Sarcolysin is D,L-α-amino-β-[*p*-di-(2-chloroethyl)aminophenyl] proprionic hydrochloride.



Synthesized in 1953 at the U.S.S.R. Academy of Medical Sciences Institute of Experimental and Clinical Oncology (IECO) (*24*), its primary effects on animals are suppression of hematopoiesis and changes in the gastrointestinal tract. Sarcolysin circulates longer than embichin in the blood of animals; although most of it disappears quickly, some of the active substance remains in the blood after 2 hours. Experimentally, sarcolysin causes total resorption of the following transplanted tumors: sarcoma 45, Walker's carcinosarcoma, Jensen's sarcoma, Yoshida's sarcoma, Danning's leukemia, and Oberling's myeloma; a number of others transplanted in mice and rats are inhibited. Sarcolysin is used in the clinic for the treatment of seminoma of the testes and its metastases, reticulosarcoma, lymphosarcoma, multiple myeloma, Ewing's tumor, angiosarcomas and, given ip, for the ascites forms of cancer of the ovaries (*10, 24, 32, 34*).

Various sarcolysin regimens are followed; 40–50 mg can be given iv every 7 days (the course dose amounts to 200–250 mg). For disseminated tumors, the shock-dose method is used in which 1.0–1.5 mg sarcolysin/kg is administered. The one-time dose, i.e., 80–120 mg iv once a month, can be successfully administered twice, according to our experience. Of 487 patients treated with the drug, 145 demonstrated some objective effect (table 3).

Asalin

Asalin, the ethyl ester of *N*-acetylsarcolysylvaline (*24, 25*), is insoluble in water. The LD50 for rats is 150 mg/kg ip and 400 mg/kg orally. Toxic doses cause suppression of hematopoiesis. Asalin, practically the same as sarcolysin in its antitumor effect, is much less active on hematopoiesis when given in therapeutic doses. When used in the clinic, it was effective against the same tumors as sarcolysin: myeloma, seminoma, reticulosarcoma, and Ewing's tumor (*24, 34*). It can be used with reduced blood indicators. The one-time dose is 0.5–1.0 mg daily or every other day; 30–40 g/course. A listing of some of the neoplasms we treated with asalin is given in table 4.

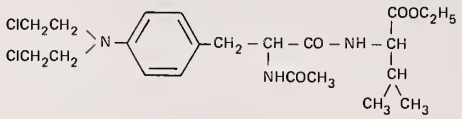


TABLE 3.—*Antitumor effectiveness of sarcolysin*

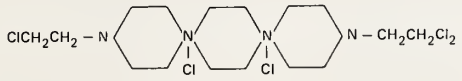
Tumor type	Number of patients	Objective effect	No effect
Lymphogranulomatosis	9	2	7
Acute reticulosis	2	—	2
Reticulosarcomatosis	59	16	43
Myeloma	47	34	13
Fungiform mycosis	1	—	1
Lymphoepithelioma	1	1	—
Bone reticulosarcoma and Ewing's tumor	40	19	21
Osteosarcoma	28	1	27
Chondrosarcoma	5	—	5
Parassal sarcoma, bone fibrosarcoma, unclassifiable osteoblastoma, osteoclastoma bone tumors	4	—	4
Angiosarcoma	26	4	22
Synovioma	7	—	7
Little-differentiated soft tissue sarcomas	14	—	14
Melanoma	12	4	8
Seminoma	42	28	14
Tumor of the testes of irregular structure	51	8	43
Cancer of the ovaries	30	21	9
Liver cancer	18	2	16
Esophageal cancer	12	1	11
Stomach cancer	34	2	32
Lung cancer	12	—	12
Others	33	2	31
Total	487	145	342

TABLE 4.—*Antitumor effectiveness of asalin*

Tumor type	Number of patients	Objective effect	No effect
Lymphogranulomatosis	3	—	3
Chronic lympholeukosis	2	2	—
Reticulosarcomatosis	9	2	7
Myeloma	11	3	8
Bone reticulosarcoma	2	1	1
Synovioma	2	—	2
Melanoma	2	—	2
Seminoma of the testes	5	3	2
Teratoblastoma of the testes with elements of seminoma	4	4	2
Stomach cancer	2	—	2
Esophageal cancer	2	—	2
Lung cancer	2	—	2
Others	2	—	6
Total	48	15	39

Spirazidine

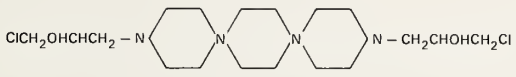
Spirazidine, N,N^3-dichloroethyl-N',N^3-dispirotripiperazine dichloride (*39, 40*), suppresses hematopoiesis when given to animals in toxic and maximum tolerable doses (MTD); fatty dystrophy of the liver and kidneys was observed in some. Experimentally, it inhibits growth of transplanted tumors (sarcomas 45 and M-1 of rats and sarcoma 180 of mice).



The compound, now rarely used clinically for the treatment of cancer of the larynx and nasopharynx in combination with radiation therapy, can be administered in a one-time dose of 20 mg. A course dose amounts to 200–300 mg.

Prospidine

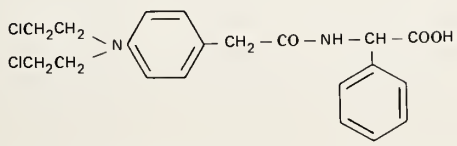
Prospidine is a derivative of dispirotripiperazine [N,N^3-di(γ-chloro-β-oxypropyl)-N',N^3-dispirotripiperazine].



In biologic study of the chloroethyl derivatives of piperazine, spirodipiperazine, and dispirotripiperazine, it was noted that their toxicity decreases and antiblastic activity rises with an increase in the number of piperazine rings. One such representative is prospidine. The LD50 of prospidine for mice, by seven-time ip administration, is 250 mg/kg; the effect is cumulative. Upon administration of toxic doses to animals, death occurs after 1–8 days. General exhaustion, decrease in size of the thymus gland and spleen, as well as suppression of myeloid sprouts in the bone marrow, are noted. Therapeutic doses cause practically no suppression of hematopoiesis. The antitumor spectrum of prospidine has been studied on transplanted tumors of rats (sarcomas 45, M-1, 536, Jensen's, carcinoma RS-1, Walker's carcinosarcoma); mice (sarcoma 180, Ehrlich's ascites tumor, lymphosarcoma L10-1, leukosis 1A); and rabbits (Brown-Pearce tumor, osteosarcoma M01). Prospidine has marked antitumor activity against many strains of rat and mouse tumors. The effect is absent only with respect to leukosis 1A, Brown-Pearce tumor, and osteosarcoma L01. In humans, the compound causes regression of cancer of the larynx and skin reticuloses. In the treatment of 67 patients with cancer of the larynx, complete regression of the tumor was noted in 26. The one-time dose is 200–300 mg, iv daily, and 4,000–5,000 mg per course. No side effects are known (*28, 38*).

Lophenal

Lophenal is *p*-di-(2-chloroethyl)aminophenacyl-DL-phenylalanine.

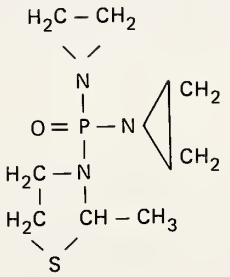


Insoluble in water, lophenal has a melting temperature of 117° C and low toxicity. The lethal dose for mice is 3,000 mg/kg and 550 mg/kg for rats. Experimentally, toxic doses cause aplasia of the bone marrow and development of agranulocytosis. The antitumor spectrum has been studied in transplanted tumors of mice and rats (sarcoma 45, sarcoma M-1, Pliss lymphosarcoma, Geren's carcinoma, and Ehrlich's mouse tumor). Lophenal has high activity against sarcoma 45.

Of 191 patients on whom the drug was tested, some positive benefit was noted in those with lymphogranulomatosis, chronic lympholeukosis, and cancer of the ovaries from the one-time, daily oral dose of 0.6–1.2 g. Thirty to 50 g are given in the course dose. Observed side effects are leukopenia and thrombocytopenia (*8*).

Imiphos (Markophan)

Imiphos, *bis*(1-aziridinyl)(2-methyl-3-thiazolidinyl)phosphine oxide, was synthesized in the Latvian S.S.R. Academy of Sciences Institute of Organic Synthesis (*15*). The empirical formula is $C_8H_{16}N_3OPS$.



The molecular weight is 233.27 and the melting point is 58–61° C. The compound (a white, crystalline powder) dissolves easily in water, alcohol, polyethylene glycol, benzene, and chloroform; it is soluble in ether (with difficulty). Upon administration of toxic doses to animals, a pattern of bone marrow aplasia develops, salivation and vomiting occur, and petechial rashes appear on the skin and mucous membranes. The clinical picture of poisoning differs little from the picture presented by the toxic effect of compounds of the ethyleneimide series, particularly, thio-TEPA. A distinctive feature of imiphos is that it suppresses erythropoiesis in therapeutic doses. Various amounts of the drug have always caused some reduction in erythrocyte content but the cumulative effects of imiphos are less pronounced than those of thio-TEPA. Thus administration of imiphos for 80 days at a dose of 14 mg/kg (0.10 LD) to white mice at 4-day intervals does not cause death, whereas when similar doses of thio-TEPA were given, all the animals died. Imiphos, studied on five transplanted tumors of rats and seven of those of mice, showed a broad spectrum of antitumor activity. The drug inhibits growth of sarcoma 45, Walker's carcinosarcoma, alveolar mucosa cancer of the liver RC-1, Jensen's sarcoma, sarcoma 180, Harding-Passey melanoma, and breast cancer and hepatoma 22 in mice, mouse rumen cancer OZh-1, and lymphosarcoma L10-1.

In clinical study, imiphos was highly active in erythremia in 99–100% of the patients (*17, 36*). Its effectiveness on various malignant tumors is presented in table 5.

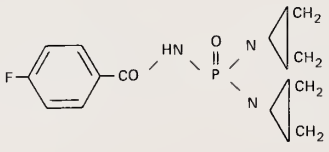
This drug should be stored in a dry, dark place at a temperature not above 5° C; activity is retained for 1 year. Immediately before use, imiphos is dissolved in 10–20 ml physiologic solution. Administered iv or im, the usual dose is 50 mg every other day; the course dose amounts to 500–600 mg. Except for some suppression of hematopoiesis, the compound is well tolerated by patients.

TABLE 5.—*Antitumor effectiveness of imiphos*

Tumor type	Number of patients	Objective effect	No effect
Erythremia (polycythemia)	107	106	1
Cancer of the ovaries	11	4	7
Sarcoma	5	3	2
Cancer of the uterus	3	1	2
Lymphogranulomatosis	2	2	—
Vascular tumor	2	2	—
Lung cancer	2	—	2
Malignant stroma	2	2	—
Breast cancer, mesothelioma, melanoma	3	—	3
Total	137	120	17

Fluorobenzo-TEPA

Fluorobenzo-TEPA, *N*-fluorobenzoyl-*n′,n′,n″,n″*-diethylenetriamidophosphate, was synthesized (*35*) at the Ukrainian Institute of Pharmacology and Toxicology. The compound is soluble in water and isotonic sodium chloride solution. Toxic doses of fluorobenzo-TEPA cause leukopenia experimentally, but red blood cells are practically unchanged. With multiple administrations of therapeutic doses to animals, the compound has a negligible effect on hematopoiesis. In antiblastic effect, it significantly inhibits growth of epithelial and mesenchymal malignant tumors (sarcoma 45, Geren's carcinoma, Crocker's sarcoma, Brown-Pearce epithelioma, lymphosarcoma L10-1). The mechanism of action consists mainly of disruption of the mitotic cycle and of nucleic acid metabolism in tumor cells.



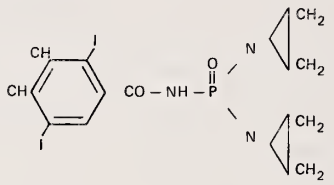
In clinical practice, fluorobenzo-TEPA is used in metastases of hypernephroid cancer of the kidneys, and cancer of the larynx and ovaries (*34*). Its antitumor activity in these and other neoplasms is given in table 6. The course dose of 400–500 mg is administered iv in 40-mg doses every other day. Since there is a delayed toxicity reaction on hematopoiesis, treatment must be stopped when a patient's leukocyte and thrombocyte counts are reduced to 3,200 and 120,000/m³, respectively.

TABLE 6.—*Antitumor effectiveness of fluorobenzo-TEPA*

Tumor type	Number of patients	Objective effect	No effect
Metastases of hypernephroid cancer of the kidneys	35	11	24
Ovarian	7	3	4
Cancer of the larynx	4	2	2
Breast cancer	3	1	2
Cancer of the parotid gland	2	—	2
Cancer of the esophagus, testes, lungs, maxillary sinus, bottom of the oral cavity, melanoma	6	—	6
Total	57	17	40

Diiodobenzo-TEPA

The antitumor compound diiodobenzo-TEPA is a derivative of ethyleneimine (2,5-diiodobenzoyldiethylenetriamidophosphate). Synthesized at the Kiev Institute of Pharmacology and Toxicology, the empirical formula is $C_{11}H_{12}O_2N_3PI_2$.



Diiodobenzo-TEPA has a broad spectrum of antitumor activity. It inhibits the growth of many transplanted tumors in mice and rats, e.g., sarcoma 45, Jensen's sarcoma, Geren's carcinoma, Walker's carcinoma, alveolar cancer of the liver (carcinoma RS), and the metastasized Brown–Pearce tumors of rabbits (*31, 35*). In the clinical study of diiodobenzo-TEPA conducted by the All-Union Chemotherapeutic Anticancer Center in 1972, researchers found it highly active in cancer of the breast, bladder, and thyroid (table 7). Leukopenia and thrombocytopenia were side effects observed in 31% of the patients.

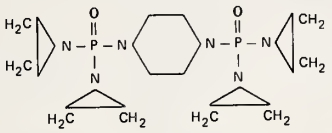
Because the compound is insoluble, 150–300 mg is given orally every other day in a course of treatment that lasts 20–25 days; approximately 2.5–3.0 g of diiodobenzo-TEPA is the total amount administered.

TABLE 7.—*Antitumor effectiveness of diiodobenzo-TEPA*

Tumor site	Number of patients	Objective effect	No effect
Breast	61	35	26
Thyroid gland	24	14	10
Bladder	20	8	12
Lungs	6	—	6
Other	22	4	18
Total	133	61	72

Dipine

Scientists at the All-Union Scientific Research Institute of the Pharmaceutical Industry (*20*) developed dipine, which is tetra(ethyleneimide)-1,4-piperazine-diphosphate.

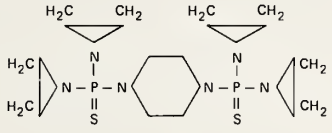


When given in toxic doses, disturbances in hematopoiesis and in the intestines develop; in MTD, the drug suppresses granulocytopoiesis. Experimentally, the growth of transplanted sarcoma 45, Flexner-Jobling carcinoma, sarcoma M-1, and Walker's carcinoma was inhibited.

Although dipine showed antitumor activity in patients with chronic lympholeukosis, metastases of hypernephroid cancer of the kidneys, and cancer of the larynx, a definitive opinion on its effectiveness as an antineoplastic drug cannot be formed until more observations have been made. Usually, 200–250 mg is administered in a course of treatment; one-time doses are 5–10 or 10–15 mg, given iv.

Thiodipine

Thiodipine, 1,4-*bis*-(diethylenethiophosphamide)-piperazine, was developed at the same institute as was dipine (*20*).



Toxic doses result in aplastic anemia and agranulocytosis, whereas moderate leukopenia has been observed after administration of MTD. Similar to dipine in several respects, it inhibited growth of transplanted sarcomas 45 and M-1. It too is used in treatment of metastases of hypernephroid cancer of the kidneys and chronic lympholeukosis. Thiodipine also suppressed growth of transplanted Crocker's sarcoma and Ehrlich's carcinosarcoma. When this drug was administered to 17 patients with metastases of hypernephroid cancer of the kidneys, an objective effect was recorded in 5. The one-time oral dose is 30 mg daily; the course dose is 600–700 mg. Leukopenia and thrombocytopenia (*34, 40*) are side effects to be noted.

Benzo-TEPA

In 1956, researchers (*35*) developed diethyleneimide benzoylamidophosphate (benzo-TEPA) at the Ukrainian Institute of Pharmacology and Toxicology. Experimental toxic doses caused gastrointestinal upsets and aplasia of the bone marrow, lymph nodes, and spleen. It inhibits growth of transplanted tumors in rats and mice (sarcoma 45, Geren's carcinoma, sarcoma 180) and the Brown-Pearce tumor in rabbits. Although benzo-TEPA is prescribed for patients with cancer of the ovaries (*18, 29, 30*), it has been used for treatment of neoplasms in other organs as well (table 8). Patients are given 24 mg iv every other day or 360–480 mg per course of treatment.

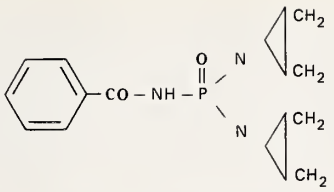
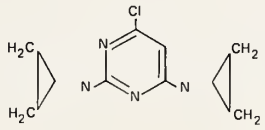


TABLE 8.—*Antitumor effectiveness of benzo-TEPA*

Tumor site	Number of patients	Objective effect	No effect
Ovaries	38	22	16
Lungs	54	20	34
Stomach and intestines	16	—	16
Other	19	6	13
Total	127	48	79

Ethimidine

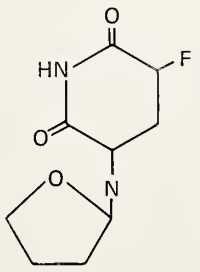
Ethimidine (2,4-diethylenimino-6-chloropyrimidine) was synthesized in 1953 by researchers at the Ukrainian Institute of Pharmacology and Toxicology, who determined that toxic doses given to animals had a cholinolytic effect and caused aplasia of the bone marrow. The antitumor spectrum of action of ethimidine was revealed experimentally in transplanted tumors of mice and rats (sarcomas 180 and 45 and Walker's carcinosarcoma).



When given in the clinic, ethimidine was effective against cancer of the ovaries and lungs (*24, 30*), but was highly toxic and caused neuritis of the auditory nerve in most of the patients, which limited its use.

Ftorafur

At the Latvian S.S.R. Academy of Sciences, Institute of Organic Synthesis, scientists developed ftorafur in 1966. An antimetabolite, the compound is one of a group of fluorine derivatives (*N'*-tetrahydrofuryl)-5-fluorouracil with a molecular weight of 200.17 and a melting temperature of 164–169° C. A white, odorless powder, ftorafur has an empirical formula of $C_8H_9O_3N_2F$.



Though similar to 5-fluorouracil (5-FU) in chemical structure, ftorafur differs significantly in pharmacologic and biochemical properties and clinical effects. For mice, the mean lethal dose (LD50) of ftorafur and 5-FU, respectively, is 1,000 and 250 mg/kg. One characteristic of ftorafur is that it penetrates the blood–brain barrier and alters the bioelectric activity of the brain. When given iv to rats, this antimetabolite circulates in the blood for at least 6 hours, in contrast to 5-FU, which disappears in 2 hours.

The spectrum of its antitumor activity is experimentally similar to that of 5-FU. Ftorafur only slightly inhibits DNA thymine and RNA pyrimidine biosyntheses in cells of certain transplanted mouse tumors. It is assumed that ftorafur is the transport form of 5-FU.

Under clinical conditions, it was successfully used for cancers of the colon, rectum, and breast (*7, 14, 34*) as shown in table 9. Ftorafur can be given iv daily, 30 mg/kg, or in a one-time dose of 1.8–2.0 g; the course dose is usually 30–40 g. Among the side effects observed are dizziness immediately after administration, suppressed hematopoiesis, and gastrointestinal disturbances.

TABLE 9.—*Antitumor effectiveness of ftorafur*

Tumor site	Number of patients	Objective effect	No effect
Stomach	73	23	50
Rectum	50	22	28
Colon	21	7	14
Breast	29	25	4
Esophagus	7	4	3
Brain	15	9	6
Other	9	5	4
Total	204	95	109

Chrysomallin

Chrysomallin is produced by *Actinomyces chrysomallus* and consists of a chromophore and two polypeptide chains. The following amino acids have been found in the composition of the antibiotic: threonine, proline, sarcolysin, valine, *N*-methylvaline, isoleucine. Chrysomallin contains three types of actinomycins: C_1 (dactinomycin), C_2, and C_3, in a 2:3:5 ratio.

Upon administration of toxic doses to rabbits, lesions of the cardiac muscle arise, diuresis is decreased, and other protein abnormalities develop in the kidneys.

When the antitumor spectrum was investigated in transplanted mouse and rat tumors, little sensitivity to chrysomallin was demonstrated; however, some effect against sarcoma 45 was observed. Other reactions induced by the compound include increased toxicity from irradiation (on the skin) and interruption of pregnancy in rats due to suppressed chorionic gonadotropin function. A therapeutic effect is considered possible for chorioepithelioma. Also, a tissue culture of Wilms' tumor exhibited sensitivity to this drug.

Chrysomallin was highly active in patients with Wilms' tumor, chorioepithelioma, and the irregular structured tumor of the testicles (table 10).

The one-time dose of chrysomallin, 500–1,000 μg, is given iv every other day; the course dose amounts to 8,000–12,000 μg. Nausea, vomiting, stomatitis, and diarrhea and, rarely, leukopenia and thrombocytopenia, can occur.

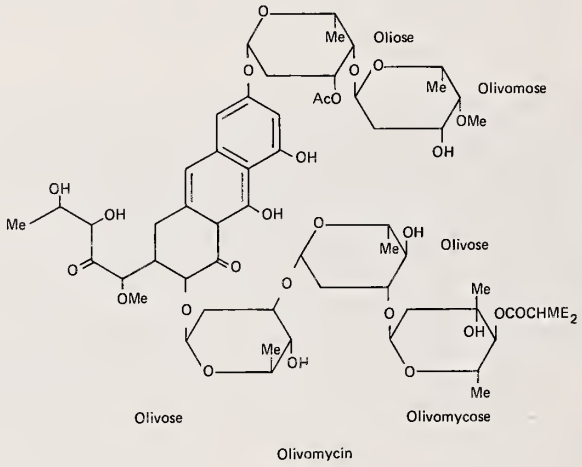
TABLE 10.—*Antitumor effectiveness of chrysomallin*[a]

Tumor type	Number of patients	Objective effect	No effect
Cancer of			
Stomach	15 (11)	—	15
Intestines	14 (10)	1	13
Lungs	13 (1)	5	8
Ovaries	98 (82)	35 (24)	63
Cervix	21 (9)	8 (2)	13
Uterus	5 (3)	(1)	4
Skin	6 (6)	—	6
Testes	62 (12)	25 (2)	37
Other malignant tumors	21 (15)	3 (1)	18
Melanoma	25 (1)	20	5
Lymphogranulomatosis	30 (21)	14 (7)	16
Reticulosarcomatosis	9 (5)	2 (1)	7
Wilms'	(29)	1 (17)	12
Other sarcomas	17 (5)	4 (1)	13
Trophoblastic disease	112 (98)	52 (46)	60
Other	7 (2)	3 (1)	4
Total	456 (310)	169 (103)	304

[a] *Numbers in parentheses* indicate the patients who received only chrysomallin; combined therapy was given to the remainder.

Olivomycin

This antineoplastic agent is produced by *Actinomyces olivoreticuli,* which was isolated from the soil in 1958 (*13*). Olivomycin, one of the glycoside antibiotics of the aureolic acid group, is 6-*O*-(*l*-olivomysyl(1 → 4)-β-acetyloliosyl)(-2-*O*-)-*l*-isobutyryl-olivomycosyl-(1 → 3)-β-olivosyl)-olivin (*3*).



Chemically, olivomycin is similar to chromomycin A3 (Toyomycin) in Japan and mithramycin in the United States. The sodium salt of olivomycin, a yellow powder that sometimes has a greenish tint, dissolves easily in water and physiologic salt solution. Infiltrates form when it is given sc to animals. Because olivomycin is poorly absorbed in the intestine, iv administration is recommended. Even with repeated injections of the MTD, it does not accumulate in animals. Toxic doses can cause infection of the kidneys, particularly of the parenchyma, with uremia developing subsequently (*4, 21*).

An increase in arterial pressure, EKG changes, and slowing of the heartbeat by 20–30% also are noted. Changes in the P, Q, S, and T spikes were variable. Olivomycin has practically

TABLE 11.—*Antitumor effectiveness of olivomycin*[a]

Tumor type	Number of patients	Objective effect	No effect
Testicles	62 (20)	32 (9)	30
Pharyngeal ring	(24)	(13)	6
Melanoma	49 (25)	8 (6)	41
Trophoblastic disease	9 (7)	5 (3)	4
Reticulosarcoma	21 (10)	3 (2)	18
Lungs	18 (15)	(1)	17
Stomach	11 (7)	—	11
Ovaries	13 (10)	(1)	12
Breast	67 (3)	—	7
Hypernephroma	6 (6)	—	6
Other malignant tumors of epithelial origin	14 (11)	(1)	13
Chronic lympholeukosis	5 (3)	(1)	4
Acute leukosis	2	—	2
Lymphogranulomatosis	4 (4)	(1)	3
Bone sarcoma	6 (4)	—	6
Other sarcomas	7 (5)	—	7
Total	294 (154)	48 (38)	187

[a]*Numbers in parentheses* indicate the number of patients receiving only olivomycin; in the remaining cases, olivomycin was used in combination with other antitumor preparations.

no effect on erythropoiesis and granulocytopoiesis, but thrombocytopenia was found in all tests (*9*).

Concentration of the antibiotic in the peripheral blood decreases quickly in the first 2 hours. About 35% of the dose administered is eliminated by the kidneys during the first 3 hours, and elimination continues for more than 24 hours.

The antitumor activity of olivomycin, studied experimentally with many transplanted tumors of mice and rats, was high and covered a broad spectrum in sarcoma 180, sarcoma 37, and strain L10-1 lymphosarcoma in mice, and Geren's cancer, Walker's tumor, sarcoma 45, sarcoma M-1, and sarcoma SSK (*27*).

According to researchers at IECO and the U.S.S.R. Ministry of Public Health, N. N. Petrov Scientific Research Institute of Oncology, olivomycin was effective against tumors of the testicles (seminoma, embryonic cancer, teratoblastoma) in the generalization stage, tonsillar tumors (lymphoepithelioma, cancer of the transitional epithelium cells), chorioepithelioma of the uterus, and melanoma (*2, 11, 12, 26, 34*) (table 11). Inasmuch as it does not have a suppressive effect on hematopoiesis, it can be used in combination with other antitumor preparations.

The usual one-time dose of olivomycin is 10–20 mg, administered iv every other day, whereas 200–300 mg is given in the course of treatment. Nausea and vomiting are observed as side effects.

Bruneomycin

In 1963 at the Institute for Research on New Antibiotics, a culture of the *Actinomyces albus* var. *bruneomycini* was isolated, from which a brown antibiotic is produced; correspondingly, it received the name bruneomycin.

Bruneomycin has acid properties; it contains two amine, three methoxyl, and one methyl group and is highly soluble in acetone, water solutions of sodium bicarbonate, and alkalis, but is poorly soluble in the lower alcohols and water. The molecular weight is 490. Data of elementary analysis permit giving it the empirical formula $C_{25}H_{22}N_4O_8$. On the basis of spectral and analytical studies of crystalline bruneomycin, its closeness to the American antibiotic streptonigrin was ascertained. A direct comparison of the physicochemical, chromatographic, and spectral properties of bruneomycin and streptonigrin confirm that these antibiotics are identical.

In a pharmacologic study of bruneomycin, Gauze (*12*) determined that its toxicity (which is a delayed reaction) suppresses hematopoiesis, hemorrhagic diathesis, and aplasia. Animals died several days after they were given the drug.

Bruneomycin has high antitumor activity according to experimental studies by Garin (*11*) and Gauze (*12*), and also by researchers who investigated its action on transplanted tumors in mice, such as strain L10-1 lymphosarcoma, strain NK/LI lymphadenosis, Fisher's strain L-5178 lymphadenosis, sarcoma 180, sarcoma 37, strain OZh 5 flat-cell cancer of the rumen, and Ehrlich's adenocarcinoma. Study of bruneomycin given iv and orally to animals with transplanted tumors has shown that 87–92% of it is bound to blood serum proteins and ascitic fluid. In the blood of rabbits, after a single iv injection of 0.5–1.0 mg/kg, it is detected for 1–2 hours; after peroral administration of 1–5 mg/kg, for 4–24 hours. Bruneomycin is found in small amounts in all organs of the animals except the tissues of the brain, which it does not penetrate. The maximum concentration in the organs is established 1 hour after iv administration and 3 hours after a peroral dose.

The drug is eliminated in the urine in a quantity equal to 1–3% of the dose given, and 0.3–1.5% is eliminated in the bile. Bruneomycin does not accumulate in the blood, which is confirmed by its uniform concentration in the blood after the first, third, and fifth daily administrations.

A study of its mechanism of action revealed that bruneomycin selectively attacks DNA synthesis and causes intensive decomposition. Although RNA synthesis is also disrupted, a much higher concentration of the compound is required. Bruneomycin interferes with that of protein considerably less than with nucleic acid synthesis. Induction of DNA degradation apparently is of an enzymatic nature, of which a strong dependence of the effect of bruneomycin on temperature is an indication. It has been shown that bruneomycin is highly lymphotrophic. This antitumor antibiotic causes atrophy of the spleen in 40–60% of the animals treated, in comparison with the controls.

Clinical use of bruneomycin was begun at the end of 1965 at IECO, where doctors found it beneficial in the treatment of neoplastic diseases of the lymph system, i.e., lymphogranulomatosis and Wilms' tumor. Of 139 patients with lymphogranulomatosis who were treated, a favorable effect (numerical rating 3, 2, 1) was noted in 104 (74.7%), but Reizinger (*37*) reported considerable benefit in 77 patients (55.4%). An advantage of bruneomycin is its activity in many symptoms of lymphogranulomatosis, especially in infections below the diaphragm. A cooperative study showed that the compound is active in other malignant tumors (*5*). Table 12 gives the results of treatment to 362 patients.

Although bruneomycin does not have cross-resistance with other antitumor preparations, it does have an effect when resistance to other agents has developed. However, bruneomycin is highly toxic and is difficult to use. Its tropism for hematopoietic tissue is harmful and results in suppression, especially of leukopoiesis and thrombocytopoiesis.

The optimum one-time iv dose is 200–400 μg every other day. The course dose amounts to 3,000–4,000 μg (2,000 $\mu g/m^2$). Orally, the one-time dose is 400 μg daily, 12,000–14,000 μg per course (6,000 $\mu g/m^2$).

TABLE 12.—*Antitumor effectiveness of bruneomycin*

Tumor site	Number of patients	Objective effect	No effect	Not evaluated
Lymphogranulomatosis	131	90	37	4
Reticulosarcoma	47	26	19	2
Chronic myeloleukosis	12	6	6	
Chronic lympholeukosis	45	25	20	
Acute hemocytoblastosis	21	5	16	
Polycythemia	5	2	3	
Wilms' tumor	25	6	9	10
Sympathoblastoma	11	2	7	2
Teratoblastoma of the testicles	6	1	5	
Soft tissues	15	2	13	
Brain	10	5	5	
Melanoma	5	—	4	1
Trophoblastic disease	4	—	4	
Epithelial tumors (lungs, stomach, esophagus, cervix, skin, breast, larynx, thyroid glands, tonsils, kidneys)	25	5	20	
Total	362	175	168	19

Rubomycin

In 1961, a culture of *Actinomyces coeruleorubidus* was isolated from soil at the Institute for Research on New Antibiotics. An antibiotic having a marked antitumor effect was produced and was given the name rubomycin. In its spectral and physicochemical properties, rubomycin is among the anthracycline antibiotics. Obtained in the form of the hydrochloride, it is soluble in water and alcohols. Chromatographic study of rubomycin showed that the antibiotic preparation consists of a number of components: A, B, and C. Component A is biologically inactive; components B and C have antibacterial and antitumor activity. Its empirical formula is $C_{27}H_{29}NO_{10}HCl$. When given sc to animals, infiltrates form, followed by necroses. Oral rubomycin is poorly absorbed. Rubomycin administered iv (the best method) is detected in the blood for several minutes and disappears 1 hour after injection. Study of its distribution in the organs and tissues revealed that 2–3 hours after an iv injection, it is found in the liver, cardiac muscle, and kidneys. Rubomycin is eliminated in significant amounts in the bile (up to 10%) and only slightly in the urine (*21*).

In therapeutic and toxic doses, this antibiotic suppresses hematopoiesis (particularly in the myeloid spur) and causes marked thrombocytopenia; lymphopoiesis and erythropoiesis are more resistant to rubomycin (*12*). With prolonged use, especially in rabbits, serious and irreversible lesions of the myocardium develop. Rubomycin is a strong immunodepressant and delays both the primary and secondary immune responses.

The drug has a broad spectrum of antitumor action. It delays development of six transplanted tumors of mice: strain L10-1 lymphosarcoma, sarcoma 180, strain OZh 5 cancer of the rumen, Ehrlich's carcinoma, NK/LI lymphadenosis, and sarcoma 37. Development of two tumors of viral origin, i.e., Rauscher's mouse leukosis and Shoup rabbit fibroma are also inhibited. Because its mechanism of antitumor action consists of suppression of DNA synthesis, it is conjectured that an antibiotic–DNA complex is formed (*12*).

In clinical use, rubomycin displayed activity in acute leukosis, reticulosarcomatosis, Wilms' tumor, and chorioepithelioma of the uterus (table 13).

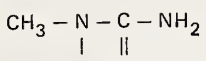
The one-time dose of rubomycin is 1.0–1.5 mg/kg administered iv every day; a second course is given after a 7-day interval. Toxic effects are manifested by suppression of hematopoiesis, nausea, and vomiting; alopecia, stomatitis, and diarrhea are observed to a lesser extent.

TABLE 13.—*Antitumor effectiveness of rubomycin*

Tumor type	Number of patients	Objective effect	No effect	Not evaluated
Acute leukosis	74	48	25	1
Reticulosarcoma	36	16	20	
Lymphogranulomatosis	6	—	6	
Trophoblastic disease	40	21	14	5
Neuroblastoma	7	4	3	
Brain	8	4	2	2
Cancer of the lungs, skin, lips, jaws, salivary glands, esophagus, cervix, ovaries; osteogenic sarcoma, rhabdomyosarcoma, sarcoma of the soft tissues, teratoblastoma of the testicles, tumor of the adrenals	24	2	22	
Total	195	95	92	8

Methylnitrosourea

Methylnitrosourea (MNU), a derivative of *N*-nitrosourea, has the following structural formula:



MNU is a yellowish-white crystalline powder with limited solubility in water; it has a melting point of 121° C. Toxic doses in experiments on animals cause aplasia of the bone marrow, spleen, and germinative elements of seminiferous tubules. The antitumor spectrum of MNU was studied on transplanted mouse and rat tumors (Ehrlich's ascites cancer, ascites form of sarcoma 37, sarcoma 180, sarcoma 45, Walker's carcinosarcoma, SSK sarcoma, breast cancer, and Harding-Passey melanoma). MNU proved highly effective in ascites tumors of mice and solid tumors of rats. Its mechanism of antitumor action is still not clear, but we know that syntheses of proteins, DNA, and RNA are delayed (*19*).

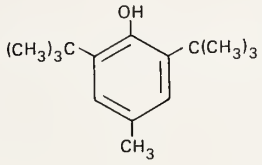
In clinical practice, MNU is used for treatment of lung cancer (low differentiation), lymphogranulomatosis, and combined with other preparations, in melanoma (*19, 33, 34*) as shown in table 14. A combination of MNU, vincristine, and dactinomycin was given in treating 70 patients with melanoma; an objective effect was noted in 35. Some patients were observed for 1.5 years without symptoms of relapse. The one-time dose of MNU is 300–500 mg administered iv twice a week; the course dose is 3–4 g. Side effects consist of nausea, vomiting, diarrhea, leukopenia, thrombocytopenia, and phlebitis. Necrosis of the tissues results if the drug is given sc.

TABLE 14.—*Antitumor effectiveness of MNU*

Tumor type	Number of patients	Objective effect	No effect
Cancer of the lungs	48	23	25
Lymphogranulomatosis	18	15	3
Cancer of the stomach	9	—	9
Cancer of the cervix	8	1	7
Reticulosarcoma	7	2	5
Cancer of the rectum	3	1	2
Cancer of the uterus	3	—	3
Cancer of the ovaries	2	1	1
Hypernephroid cancer	2	—	2
Cancer of the breast	2	—	2
Cancer of the vulva	2	—	2
Mesothelioma	2	—	2
Malignant chordoma	2	—	2
Acute leukosis	3	—	3
Myeloid disease, chronic lympholeukosis, cancer of the nasopharynx, cancer of the colon and bladder, leukomyosarcoma of the stomach, chondrosarcoma, sarcoma of the soft tissues, teratoblastoma of the mediastinum	9	—	9
Total	120	43	77

Ionol

This compound, 2,6-di-*tert*-butyl-4-methylphenol, is an inhibitor of free radical processes. In the precancer and cancer stages, there is a significant increase in concentration of free radicals, in comparison with that in homologous normal tissues. Experimentally, simultaneous use of a carcinogen and ionol delayed tumor development, so its use clinically was tested. Ionol is now given for cancer of the bladder (*41, 42*).



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Experimental Selection of Antitumor Compounds: Products of Synthetic and Vegetable Origin

Z. P. Sof'ina[1]

Systematic experimental study of antitumor activity of chemical compounds was begun in the U.S.S.R. at the end of the 1940's, when the laboratories of experimental tumor chemotherapy were established in Leningrad (N. N. Petrov Institute of Oncology) and in Moscow (Ordzhonikidze All-Union Scientific Research Chemical–Pharmaceutical Institute). Similar work developed in the Ukrainian Scientific Research Sanitary–Chemical Institute and in the U.S.S.R. Academy of Medical Sciences, Institute of Experimental and Clinical Oncology (IECO). In subsequent years, the number of institutions concerned with experimental tumor chemotherapy gradually increased in the Soviet Union, and now there are more than 20. Each has its chemistry branch and established area of research.

It should be emphasized that, from the beginning, the work of finding antitumor compounds in the U.S.S.R. was conducted rationally, i.e., substances synthesized according to a definite plan were studied in the development of one or another hypothesis. This concerned not only potential antimetabolites but also alkylating compounds, the structures of which were conceived beforehand (*47*). To a great extent, this determined the choice of methods of experimental selection of antitumor compounds in the U.S.S.R.

Since this approach proposes testing a relatively small number of compounds of a specific structure, the investigators did not have to produce single models or model systems, sensitive to compounds with various chemical structures and mechanisms of action. Most likely, each group of research chemotherapists dealt with a relatively small number of types of compounds and restricted themselves to experimental models sensitive to a given group of compounds. At the same time, the testing of those substances developed by specific hypotheses led to more detailed, comparative study, including elements of investigation of their mechanisms of action (*10, 43, 84, 85*).

Only in recent years, when the search for anticancer compounds was extended to the antibiotics and products of plant origin, and the necessity for examining a larger number of compounds of different chemical classes became more urgent, have the chemotherapists of the Soviet Union been faced with the problem of creating optimum and economical selection systems and deciding the problem of its unification with greater caution. With respect to the necessity for unification of the selection system, there is no consensus among the various investigators.

Continuous efforts have been made to optimize testing of the antitumor activity of compounds. Because of this, models and methods of study of potential anticancer compounds were reviewed repeatedly in different institutions of the U.S.S.R. The development of a system of experimental study of antitumor compounds at IECO can serve as an example.

In the 1950's, researchers at IECO investigated the antitumor activity of compounds on three strains of transplanted tumors: mouse sarcoma 180, Ehrlich's tumor, and rat sarcoma 45. This system was adopted by the Second Coordinating Conference on Tumor Chemotherapy in 1958 (*43*). However by 1960, G. L. Zhdanov (*29*), considering in detail methods of selection of antitumor compounds, recommended that a primary selection be made on a different set of transplanted tumors: sarcoma 45, Walker's carcinosarcoma 256, and sarcoma 180.

For several years, all interesting compounds were tested on a large set of experimental tumors as well as the obligatory tumor strains adopted for primary selection. The number of models for certain preparations reached 20–25 and included different strains of transplanted and induced tumors of mice, rats, and rabbits (*62, 81*).

The results of special studies performed in the 1960's caused the system of primary selection in the Institute to be reviewed. S. S. Bokaeva (*6, 7*) simultaneously made a comparative study of 13 compounds with previously established antitumor activity on 20 transplanted tumors of mice, rats, and hamsters. The advisability of testing new compounds on tumors having moderate and differing sensitivity (which is important) to compounds of one and the same class was demonstrated. In particular, the author recommended the use of rat sarcoma 45 and mouse sarcoma 298 (C57B10) in the selection of alkylating drugs.

Later, the work of M. Kh. Karaivanova (*36*) and other colleagues in the Laboratory of Experimental Chemotherapy (IECO), served as a basis for a differentiated method of initial selection of various classes of compounds (*45*). Testing alkylating agents began on mouse polymorphic cell sarcoma 298 and on two rat tumors: spindle-cell sarcoma 45 and Walker's carcinosarcoma 256. Compounds with supposed antimetabolite action were investigated on mouse breast adenocarcinoma 755 (C57BL) and selected mainly on squamous cell mouse forestomach cancers PRG(CC67Nt) and OG5 (C3HA) (*60, 73*). The route of administration also was changed from that adopted earlier.

Compounds displaying activity in the initial selection were studied further on other experimental models to discover differences from well-known antitumor compounds of similar structure, both as to effect on the tumor spectrum and on normal tissues and organs, mechanism of action, etc.

At the present time, the selection system at IECO is being reviewed again and will be detailed later.

The antitumor compound selection systems in other laboratories of experimental chemotherapy in the Soviet Union have undergone specific, although considerably fewer, changes. The Ordzhonikidze All-Union Scientific Research Chemical–

[1] Head, Laboratory of Experimental Chemotherapy, Cancer Research Center, Moscow, U.S.S.R.

Pharmaceutical Institute presently recommends use of the transplanted rat tumors sarcoma 45, sarcoma J-1, sarcoma 536, Geren's carcinoma and tumors, sarcoma 180, sarcoma AK, sarcoma M-49, Ehrlich's tumor, carcinoma NK, as well as leukosis L, for initial selection (*21, 85*). In the Ukrainian S.S.R. Academy of Sciences, Institute of Problems of Oncology, Geren's carcinoma, sarcoma 45, and sarcoma 180 are used for initial selection of new preparations if alkylating agents are being tested, and Walker's carcinoma 256, sarcoma 180, and Schvets erythromyelosis, if antimetabolites and antibiotics are being studied. In selection of antileukosis compounds, several transplanted leukoses are used [La, Lel, TsOLIPK-8, etc. (*9, 10*)]. In those institutions conducting experimental chemotherapy, other sets of such transplanted tumors are used.

We consider that the absence of a single anticancer compound selection system has advantages and shortcomings. One advantage is that a more differentiated approach to testing each compound is possible. Under such conditions, the biologist readily takes note of all characteristic features of a given compound (structure, possible mechanism and direction of action, physicochemical properties, etc.), and discloses some new aspects of its action as early as the primary stages of the research. This becomes particularly important in work with compounds of complex chemical structure, in which minimal structural changes lead to a highly significant deviation in biologic action. Such deviations cannot be found in standard research methods but only with detailed study of specific systems (special types of tumors, normal tissues or systems, in biochemical tests, etc.). It is much more complicated to study all these factors by working on a restricted framework, which naturally cannot be sufficiently exhaustive.

On the other hand, the absence of a single selection system makes a comparison of compounds impossible and complicates correlation and conclusions on the advantages or disadvantages of a series of compounds or an individual compound.

It seems that the solution to adoption or rejection of a single selection system by countries, or institutions, or an individual institute or laboratory should be determined by the purposes which are pursued in each case. For example, if the decision of examining as large a number as possible of different, especially random, compounds is made, then testing their activities in a strictly established schema is not practical. From this point of view, the antitumor compound selection system in the National Cancer Institute (U.S.A.) appears to be completely justified; up to 50,000 new and mostly random compounds are tested there each year (*97, 107*). The known loss of some portion of an active compound is compensated for by the possibility of discovering a larger number of new and interesting structures, if the selection system chosen does not limit the group of compounds found to preparations with only monotypic mechanisms of action.

Work with similar analogues of a specific active compound should be organized differently; the basic purpose of the research is the development of fine distinctions between members of a series.

Work has always progressed precisely in this manner in most of the corresponding institutions of the Soviet Union and, especially at IECO (*43*). For example, in the study of alkylating agents containing residues of various biologically active compounds in their molecules (specific proteins, hormones, nucleic acid components, etc.), not only is their antitumor activity tested on a certain set of tumor lines, but the role of the "carrier" of the cytotoxic group (*62, 82*), effect on dependent tissues (*77*), and other actions have been examined. Selectivity of antitumor action of compounds structurally similar (*29, 30, 35, 42*) and various manifestations of side effects have been studied comparatively and in detail. Antifolic preparations are tested on the appropriate enzyme systems. Provisions are made for other aspects of research, e.g., for false negative responses. False positive responses should be kept to a minimum, and, although this error can be corrected by subsequent study of the compound, overloading subsequent stages of the selection is undesirable. That the initial selection system be highly reproducible, simple, economical, and readily responsive is important.

Since tumor chemotherapy became possible, the attention of investigators has been turned many times to various methods of testing compounds in vitro. Attempts have been made to use plants, microbe systems, steady-state or primary tumor-cell cultures, various biochemical systems, etc. However, as of now, no in vitro system has been found that would give a sufficiently high correlation with clinical data and would eliminate studying antitumor activity of the compounds in vivo. It should be recognized that certain methods of testing drugs in vitro give highly significant information on their biologic activity and certain aspects of their mechanism of action. Of course, such information must be considered in an overall evaluation of a compound and in the decision on the subsequent fate of the compound.

Thus tumor cell cultures are used extensively in various countries; the most widespread and accepted among them at present is the KB cell system (human cancer of the larynx). According to Geran et al. (*95*), this cell culture gives the greatest coincidence with results obtained on L1210 leukosis in vivo.

As a rule, tissue cultures are not used in the Soviet Union for selection of antitumor compounds. However, efforts are constantly being made to adapt certain tumor cell cultures to this purpose. Thus scientists at IECO, in parallel with in vivo tests, are examining the effect of preparations on HeLa cultures (*83*), and cultures of tumor cells in the human ovary (CaOv) now are being studied similarly (*23*). A definite correlation has been found with results obtained in in vivo tests. A much lower correlation was detected between microbiologic systems and tests in vivo (*112*). There apparently may be greater interest in the method of testing compounds with supposed antitumor activity on microbe mutants, developed by A. Z. Smolyanskaya and F. I. Solodovnik of IECO (*76*).

One method of examining compounds in vitro that appears highly promising is that involving antitumor activity in primary cultures of suspended ascites tumor cells, developed by L. B. Ivanitskaia and L. V. Makukho (*34, see* the corresponding chapter in this monograph). They discovered 20% active among 100 actinomycete cultures examined. Subsequently, 65.3% of them proved to have antitumor activity in vivo. This method was approved by the Laboratory of Experimental Chemotherapy at IECO and certain other institutions.

Despite all the attractiveness of selecting antitumor compounds in vitro, tests on tumor-bearing animals have the greatest appeal, as confirmed by the increased number of reports dealing with antitumor activity of drugs on transplanted tumors of mice and rats.

Experience in experimental tumor chemotherapy shows that there is not a single antitumor compound used in the clinic that was not active in some of the transplanted animal tumors; use of the latter for selection of anticancer compounds is fully substantiated. At the same time, it is well known that various transplanted rodent tumors have sensitivities to certain compounds that differ greatly from one another in their mechanisms of action (*44*). Therefore, the choice of suitable models of

this kind for revealing active antitumor compounds is an important but complicated affair.

We have a transplantable tumor bank composed of foreign tumor strains and those produced in the U.S.S.R. now numbering about 70, half of which are Soviet (*56, 66–68*). However, only a limited number are used for initial tests of antitumor activity of substances.

Geren's carcinoma, sarcoma 180, sarcoma 37, sarcoma AK, Ehrlich's tumor, and ascites tumor NK/LI have been the most widely used until recently in our laboratories and are still used in some (excluding the Laboratory of Experimental Chemotherapy at IECO).

The initial selection usually is made on 2 or 3 tumors, but choice of tumor strains is determined primarily by sensitivity to the class of compounds being examined (*9, 45, 46, 85*).

IECO has adopted the following system for initial selection of antitumor compounds in vivo. All compounds undergo tests in mice with leukemia L1210, mammary adenocarcinoma 755, and Lewis lung tumor. Compounds of vegetable origin, especially before the stage of obtaining the pure drug, are examined in mice with PRG and OG-5 squamous cancers of forestomach (PRG is preferred).

The following factors are considered before selection is finalized:

1) The system is composed of tumors having different kinetic indices (proliferative pool, volume doubling time, duration of phases of the cell cycle, etc. (table 1).

2) The system is composed of tumors differing in sensitivity to known antitumor compounds, and the selected strains supplement one another, so that no active preparations of various classes are omitted (*98, 105, 107*).

3) The system includes tumor strains universally used, making possible comparison of results obtained here with data of other investigators (in particular with the United States, with whom we have signed an agreement on the conduct of joint research in the field of cancer chemotherapy).

4) The selected tumors have moderate sensitivity to antitumor compounds to facilitate comparison of compounds, including those similar in structure but differing in strength of the antitumor effect.

5) The experiments are performed on mice to reduce the number of compounds necessary for making a selection (in comparison with experiments on rats).

6) The tumors mentioned above are carried in inbred mice guaranteeing more syngeneity of the system (*38*). The advisability of using only syngeneic tumors for selection is known and does not require additional justification. Nevertheless, for a better understanding, we cite one example of an investigation of antitumor activity of a compound (selected at our laboratory 10 years ago) with outbred animals and nonsyngeneic tumors (*72*). 5-Oxytryptamine (serotonin), isolated as a vegetable alkaloid, inhibited growth of mouse sarcomas 37 and 180 and also caused regression of Jensen's rat sarcoma, but it was not significantly toxic in vitro on tumor cells (*74*). Clinically, the preparation did not affect human tumors, and later we found that it was ineffective on syngeneic mouse tumors as well (except hormone-dependent ones). Recently, Japanese experimenters, in a study of the mechanism of antitumor action of serotonin and certain polysaccharides, demonstrated that inhibition and regression of sarcoma 180 occurs and is mediated through immune systems (*99, 102–104*). For treatment in humans, the compounds selected need act only on the syngeneic tumors. Therefore, it is important in making the selection that the antitumor activity of all compounds on syngeneic tumors of inbred mice be correctly evaluated.

In screening some lesions, we consider it to be not only permissible but useful to include among the models one or two nonsyngeneic ones, e.g., sarcoma 37, sarcoma 180, or Walker's carcinosarcoma, in addition to the obligatory set of syngeneic tumors. Moreover, as cancer immunotherapy develops, one can expect that immunoactive compounds, which are easier to select in nonsyngeneic models, will be used more frequently for patients.

We do not consider that the model system presented here is the only possible one; other systems that would meet the same requirements are viable. However, we doubt that any of the existing systems could be called "ideal"; any can and must be improved. Therefore, we continually attempt to study new models, which, with the rapid accumulation of knowledge and changes in methods and equipment, is necessary to keep abreast of all the on-going research.

Thus at IECO, several highly differentiated mouse tumors are being studied: PRG squamous cell cancer of the forestomach of C57Br mice (*24*), AKATOL adenocarcinoma of the large intestine of BALB/c mice (*33*), and RShM cervix cancer of CBA mice (*1, 2*). PRG is attracting attention because of its sensitivity to substances that differ sharply in their mechanism of antitumor effect from known ones (chanerol, chanerosan) (*73*). AKATOL has little sensitivity to most tumor preparations; however, it reacts to endoxan, vincristine, chanerol, and, to a slight extent, 5-fluorouracil (5-FU). RShM is a hormone-sensitive tumor, and is similar to human cervix cancer in its properties, according to the data of the Laboratory of Endocrinology and Experimental Chemotherapy.

Definite attention also is being given to certain strains of leukoses, which differ in sensitivity from L1210, such as La(C57B1 mice), P388 (DBA/2 mice), and MOPC-406 (BALB/c mice).

At IECO, considerable emphasis is given to work on the biochemical characteristics of various strains of transplantable tumors, since their sensitivity to an antitumor compound is determined, not only by the growth rate, but, to a greater extent, by peculiarities of their metabolism.

The question of the scheme of application in tests and the

TABLE 1.—*Certain kinetic parameters of L1210 leukosis, Ca 755, and Lewis lung cancer*[a]

Tumor	Average doubling time, days	Labeling index, %	Duration of phases, average hr				
			T_c	T_s	$T_{g_2}^+$	T_M^+	T_{g_1}
L1210	0.4	65	10	8.6		1.4	
Ca 755	1.6	18	15	6		9	
Lewis lung cancer	2.9	15	19	9		10	

[a] *See (110).*

criteria by which the presence or absence of activity is judged, is highly significant in selection. As new and more effective compounds appear, the requirements for activity have increased. Whereas, in the early years of modern tumor chemotherapy, the mere presence of antitumor activity was generally considered sufficient for clinical testing, at the present time, a considerably higher level of antitumor effect, a specific selectivity of action, and a difference from known antitumor compounds are required for a new preparation to be recommended. Previously, proven growth inhibition of a tumor with high sensitivity to a given class of compounds was considered sufficient. Now the criterion of activity is either *cure* of animals with a sensitive tumor or *significant inhibition* of the growth of slightly sensitive tumors. More and more often, the life-span of treated animals, in comparison with untreated ones, is used as a criterion of effectiveness applicable to solid tumors.

During the past few years at IECO, in a study of antitumor activity of alkylating compounds, the inhibition of growth of sarcoma 298 (which is highly sensitive to this class of compounds) by 75% was considered to be sufficient for selection (*46*). Then the activity criterion became the percent of regressed tumors. At the present time, sarcoma 298 is excluded from the set of strains on which initial selection is made. The activity criteria of this group of compounds on tumors moderately sensitive to them, i.e., adenocarcinoma 755, leukemia L1210, and Lewis lung tumor are: Either the increase in life-span is raised by at least 25% or tumor growth is inhibited more than 50%. The effectiveness of preparations in the antimetabolite or vegetable compound groups (if the latter are purified to the individual compound) is evaluated similarly. Only when testing plant extracts or products obtained at various stages of isolation of the active principle are lower activity criteria used, e.g., the presence of a statistically significant and reproducible tumor growth inhibition with tolerable doses.

As a result of recent research, the following test system was adopted in the laboratory. Animals receive drugs five times daily, with the initial treatment given 24 hours (L1210) or 48 hours (Ca 755, Lewis lung cancer) after tumor transplantation. An evaluation of the effectiveness of the treatment is made by life-span of the animals or by percent tumor growth inhibition, determined 1 week after stopping treatment with the maximum tolerable dose. The percent inhibition is calculated on the basis of tumor weight or by their conventional volume, which is the product of the values of three mutually perpendicular diameters of the tumors.

While performing a special investigation on the sensitivity of transplanted rat and mouse tumors to various known antimetabolites, N. A. Vodolazskaia demonstrated that it is best to begin treatment 48 hours after transplantation (*50*). By this time, the tumor cell "take" has ended in almost all the animals and the formation of a tumor node has begun. Beginning the treatment later reduces the effect, and subsequent discovery of active compounds is impaired. A dose of 30 mg 6-mercaptopurine/kg daily inhibits growth of hepatoma 22 by 60% if given 48 hours after the tumor is transplanted, but if the interval is increased by 24 hours, the antitumor effect is reduced to 30%. A similar result is obtained when mice also bearing this tumor are treated with 15 mg 5-FU/kg daily; tumor growth is inhibited by 70 and 50% when therapy is administered 48 and 72 hours, respectively, after transplantation.

Karaivanova (*36*) also noted a sharp reduction in effect by a late start in drug administration, even in the treatment of tumors highly sensitive to the compound. For example, although sarcolysin cured 80% of the rats with Walker's carcinosarcoma 256 when treatment was given 48 hours after transplantation, a 4-day delay produced resorption of only 10% of the tumors.

Since the principal objective of initial selection is the establishment in principle of the presence of antitumor activity in a compound, we consider it advisable to create those conditions that would promote effect in the first stage of examination.

That short courses of treatment were possible and effective was further substantiated by Karaivanova and others of our laboratory. They used 22.5 mg sarcolysin/kg as therapy for sarcoma 45 (the tumor is highly sensitive to this compound) and reported 95% tumor growth inhibition and a cure of 22% of the animals in a 5-day course but only 80% less growth and no cures in 10 days of treatment. This pattern is repeated with other tumors and compounds having different mechanisms of action. Another example that can be cited is that of Ca 755, which is only slightly sensitive to 5-FU (12% growth inhibition). No antitumor effect was observed in a 10-day course of treatment, whereas growth was inhibited by 55% in 5 days.

We would not advise shorter courses of treatment in initial selection, although eventually, in the development of optimum treatment schedules, we may find that some compounds should be used in short shock courses or one-time administrations. Similar conclusions have been reached in other institutions of the Soviet Union (*22*).

Numerous active compounds display no less and, sometimes, higher antitumor activity, when they are administered at intervals of 3–4 days, and 2–3 injections in all are sufficient to display activity (table 2). In the presence of a sufficient amount of the test substance, an initial choice can be made with both treatment systems.

As has been shown by many years of experience of scientists at the chemotherapy laboratory (IECO), results should not be evaluated directly after completion of a course of administration of the compound (*36*). The evaluation is more nearly correct if it is done 7–10 days after treatment has ceased, especially if the 5-day course of administration was followed. During these 7–10 days, delayed toxicity of the compound, which would require a subsequent change in dose, may be revealed. Also, the antitumor effect is readily observed. However, the activity disappears if tumor growth inhibition was weak and temporary, and it increases if the compound causes the lesion to regress (table 3).

We now consider that observation of test animals for 7 days after completion of a course of treatment is sufficient, since it is just at this time that massive death from an overdose (95%) ends. This time is the most suitable for estimating the results of treatment, especially if the tests are done on tumors of low sensitivity (table 4).

With due consideration of our data, that of other tumor chemotherapy laboratories in the Soviet Union, as well as that of the National Cancer Institute in the United States, we deem it unnecessary, by preliminary trials on intact animals and antitumor compounds, to determine toxicity of the drugs. Usually, despite a large amount of the compound and considerable time expended in such an investigation, little concrete information on the dosage to be used in therapeutic tests is obtained. More information on antitumor and toxic activity can be acquired if the drug is given at various dosages to several groups (3–5) of tumor-bearing animals. Obligatory repetition of the tests confirms the data and suggests corrections in dosages. A detailed determination of toxicity would be performed during the special toxicologic phase that all active antitumor compounds undergo. In the initial selection, we con-

TABLE 2.—*Effect of compounds on tumors by different schedules of use*

Tumor	Compound	Dose in mg/kg/interval between administrations in hr × number of administrations	Tumor growth inhibition in percent (7 days after stopping treatment)
Ca 755	Dactinomycin	0.03/24 × 5	0
		0.12/96 × 2	59
Lewis lung carcinoma	5-FU	25/24 × 5	63
		65/96 × 2	70
	Sarcolysin	2/24 × 5	59 (0% increase in life-span)
		7/96 × 2	64 (30% increase in life-span)
	Cyclophosphan	40/24 × 5	83
		100/96 × 2	100
AKATOL	Dactinomycin	0.03/24 × 5	35
		0.12/96 × 2	53
	Vincristine	0.2/24 × 5	38
		0.6/69 × 2	70

TABLE 3.—*Antitumor effect of compounds, evaluated at various times after stopping treatment*[a]

Tumor	Compound	One-time dose in mg/kg and number of administrations	Percent tumor growth inhibition after stopping administration of compound		
			On following day	After 5 days	After 10 days
Sarcoma 45	Sarcolysin	4 × 5	70	99.5	100
Walker's carcinosarcoma 256	Benzo-TEPA	5 × 5	98	98	100
"	Nitrogen mustard	0.18 × 5	66	94	97
"	Cyclophosphan	20 × 5	100	100	100
Ca 755	6-Mercaptopurine	20 × 10	99.3	100	
Sarcoma 180	5-FU	14 × 10	54	40	
Pliss lymphosarcoma	Methotrexate	0.1 × 10	98	96	

[a] *See (36).*

TABLE 4.—*Antitumor effect of various compounds on Lewis lung carcinoma*

Drugs	Dose in mg/kg daily, 5 days[a]	Percent tumor growth inhibition after completion of treatment		
		After 24 hours	After 7 days	After 10 days
Cyclophosphan	40	96	83	65+[b]
Sarcolysin	2	20	59	36+
TIC-mustard	60	21	56+	37
Fluorodopan	18	66	68	62
Chanerol	10	65	54	43
Cyclocytidine	120	50	35+++	13
DTIC	50	0	26	14

[a] Treatment was begun 48 hr after transplantation and continued 5 days.
[b] + = Death of animals at this time.

sider all compounds that meet the criteria for effectiveness to be active.

At IECO, the first stage of experimentation of antitumor activity of new drugs is done most often in vivo in a system consisting of three transplanted tumors, and of tests of their biologic activity in various systems in vitro. Based on the results of these studies (data obtained in vivo are decisive), the presence or absence of antitumor activity of a given compound is established. Compounds recognized as active are transferred to the profound study phase.

Antitumor compounds in other laboratories of the U.S.S.R. are selected in approximately the same manner, with differences only in the sets of transplanted tumors used and certain details in testing procedures.

The extent to which a compound is investigated depends on its structure. Compounds of new chemical classes, not previously encountered among the known antitumor compounds, can be studied in less detail. Some of them can be relegated immediately to toxicologic and pharmacologic investigation and, subsequently, safety of administration to man is assured.

The remainder are studied according to a general plan, but usually less rigid requirements are placed on them than on those belonging to known groups of antitumor compounds.

At present, before any new agent is recommended for therapy in humans, its advantages over known compounds must be demonstrated. The second stage of investigation is devoted to elucidation of these advantages. What are some of the properties considered as improvements over known drugs? They can consist of an effect on other forms of human tumors than those subject to chemotherapy at a given time, or of another spectrum of effect of the antitumor compound, or they may be active on a tumor that was drug resistant, i.e., tumors that could not be controlled by previously effective compounds. Advantages of new drugs may also consist of greater selectivity of antitumor effect than old ones, inducing a therapeutic reaction without, or with considerably less, injury to normal tissues and organs. Solubility and possible administration directly into the circulatory system or specific cavity of the body, penetration through one tissue barrier or another, the possibility of producing the necessary medicinal forms, and the cost of the preparation, can prove advantageous.

Because problems of toxicologic and pharmacologic study of antitumor compounds, as well as investigation of their biochemical mechanisms of action, are presented elsewhere in this monograph; we will not discuss them here.

The results of any medicinal preparation depend to a great extent on the *frequency* of its use, *route of administration,* and *medicinal form* in which it enters the body; if and when any biologic effects appear may depend on its correct application. Therefore, if a drug is suggested for in-depth study, the optimum schedule of its application and the necessary medicinal form that are used in subsequent experimental investigation of the compound, must be determined.

For example, in tests with phenestrol (4,4′-*bis*-[di(2-chloroethyl)aminophenacetyl]synestrol), it was shown that the compound can only be administered sc; because its ester bonds are cleaved, other routes of administration would cause a loss of the specific biologic effect of the compound (table 5). All efforts were directed subsequently toward obtaining the appropriate medicinal form of the compound, despite the nature of the complex work. In the in-depth study that followed, the preparation was administered only sc.

TABLE 5.—*Duration of estrus in ovariectomized female rats upon administration of phenestrol sc and orally*

Drug	Dose, μg[a]	Route of administration	Duration of estrus, days
Synestrol	100	Sc	4
"	"	Oral	2
Phenestrol	300	Sc	>45
"	"	Oral	2[b]

[a] Synestrol and phenestrol doses are equimolecular.
[b] As the duration of estrus indicates, phenestrol is decomposed, with release of an equimolecular amount of synestrol, the action of which is disclosed in this case.

Some examples of optimum treatment schedules of tumors with selected antitumor compounds developed at IECO include the study of sarcolysin and its derivatives, fluorodopan, chanerol, and others. As a result of these studies, it was deemed more advisable to administer some compounds at longer intervals in massive doses (*11, 16, 27, 28*), whereas others that produce a greater antitumor effect by frequent administration should be given in smaller amounts.

However, one should remember that any highly active antitumor compound that meets the criteria for clinical testing requires that optimum treatment conditions and medicinal forms be ascertained after toxicologic investigation of the compound, with data appropriately noted (*see* Syrkin's paper, "Preclinical Study of Antitumor Compounds").

Among the most complicated problems facing research chemotherapists is the determination of the spectrum of antitumor effects of a compound. Despite vast amounts of research data accumulated everywhere and the relatively high correlation existing between experimental and clinical observations, a distinct correlation has not been established in the realm of sensitive, transplantable tumors. Only in isolated cases can a biologist, with a fairly high degree of confidence, recommend that a clinician test a drug on specific types of human tumors.

Generally speaking, we believe that highly differentiated, induced animal tumors are most suitable for use in determining the spectrum of antitumor effect of drugs. Unfortunately, conducting a massive experiment on these models is difficult because tumors are not uniform and their growth rates vary. Hence the selection of experimental groups is complex. These complications increase the cost in time and money of any investigation. Despite these hazards, induced rat mammary gland tumors (*3–5*), skin cancers (*100, 113*), virus-induced (*65*) or spontaneous virus leukoses (*110*) have been used successfully. Spontaneous tumors (*59*) and those in the first generation (passage) after transplantation of induced or spontaneous types are also examined. Foreign and domestic scientists have demonstrated that during early (approximately 2–4) generations, initial characteristics (differentiation, response to an action) similar to those of the original tissue are retained, a factor that makes them suitable for determination of the scope of antitumor effect a chemical may possess.

Later in the third to fourth generation (passage), specificity changes sharply, and the lesions resemble other transplanted neoplasms, with greater and greater loss of their inherent characteristics. At IECO, N. D. Lagova (*40*) demonstrated that the first generations of spontaneous breast cancer of rats (RMK-1) were highly sensitive to estrogens. Synestrol caused 95% inhibition in tumor growth. However, by the tenth generation, this sensitivity was lost. Similarly, by transplantation, the hormone sensitivity of induced rat ovarian tumors (*12, 13*), mouse uterine cancer (*1*), and mouse adenocarcinoma of the large intestine to 5-FU, etc., was lost.

Although use of a set of transplanted tumors for study of the spectrum of action continues, if differences in effect on the spectrum of animal tumors are found, only a general conclusion usually is drawn on the supposed difference in man (*8, 43, 80*). Such conclusions obviously should be drawn with utmost caution. An extensive set of transplanted tumors has been used for a long time at IECO for this type of study on a large group of alkylating agents, and a series of compounds with statistically significant differences in suppressing tumors were selected for clinical testing. However, during their study in the clinic, significant differences could not be found among individual compounds that were structurally similar (sarcolysin and its dipeptides, asalin, asaphan, and asaley) in their spectrum of action. At the same time, definite differences were found between the chloroethylamine derivatives having no greater difference in spectrum of action on experimental tumors, but simultaneously more significant variations in structure of the alkylating group carrier (sarcolysin and dopan) (*48, 62, 81*).

Investigators were attracted for a long time to heterotransplants of human tumors as models (*14, 18, 19*). Until recently,

transplantation of human tumors to animals could only be done under conditions of artificial suppression of immunity by factors that significantly change the reactivity of the animal and, of course, cannot show up in the response of the tumor to the treatment. However, in relation to development of the diffusion chamber method (*25, 26*) and creation of lines of athymic mice (*96, 106*), the possibility of transplanting human tumors to animals, without additional actions (though these conditions are not completely analogous to those taking place naturally) has been raised. These models have generated the hope that many of the problems related to the spectrum of antitumor activity can be solved.

Many chemotherapists expect that development of biochemical profiles of human and animal tumors could assist in the orientation of lesion sensitivity to antitumor compounds.

The possibility of suppressing tumor growth with emerging drug resistance should be considered as a great advantage of a chemical compound. In the second phase of new active compounds, a definite place should be given to 1) investigation of how drug resistance to these compounds originates, 2) problems of cross-resistance with known preparations, and 3) the problem of overcoming resistance of tumors that is acquired during treatment.

These problems have been and are being studied in the Soviet Union, mainly at IECO and, more recently, at the Kazakh Scientific Research Institute of Radiology and Oncology (*17, 31, 32, 86–89*). In these institutions, the conditions in which drug resistance arises with the greatest regularity were determined, and a number of strains resistant to various compounds were obtained. These strains are now used in chemotherapeutic experiments, in biochemical studies of the nature of drug resistance, and in assays of the molecular mechanisms of action of the antitumor compounds.

A most important aspect of the second stage of compound selection is the *selectivity of antitumor action*. Usually, selectivity of antitumor action is expressed by the ratio of the size of dose causing a specific level of toxic effect (maximum tolerable dose, median lethal dose LD10, LD50, etc.) to the size of doses causing a specific degree of antitumor effect (ED50, ED90, etc.). However, even if the differing sensitivity of the body to a preparation is disregarded and it is considered constant, the second value can change radically depending on which tumor has been affected. Therefore, one and the same preparation can have completely different indices of selectivity of action in tests on different tumors, because their sensitivity is variable (*29, 30, 32, 42, 56*). Comparison of compounds by these criteria should be objective: in complete coincidence of the spectra of their antitumor action or for confirmation of a conclusion as to the lesser or greater effect of one compound or another on a specific tumor.

In the Soviet Union as elsewhere, selectivity of antitumor action is expressed by various indices. Those proposed by L. F. Larionov [LD10/ED90; LD10/ED60; (*42, 43, 45, 46*)] and G. L. Zhdanov [LD50/ED50; (*29*)] have been used here to evaluate antineoplastic drugs.

However, any reference to these and similar indices, including those used abroad (*91–93, 101, 108*), should be made cautiously; otherwise, their use can lead to serious errors. If the LD50/ED50 ratio for sarcolysin, obtained on sarcoma 45 (*71*) is compared with the corresponding index of dopan (*48*), the conclusion would be drawn that dopan has less selectivity of action; dopan would be rejected as having little promise, comparatively (*29*). It is well known that these compounds display activity in different forms of malignant neoplasms in man and therefore cannot be compared with one another.

A comparison of sarcolysin and cyclophosphan chemotherapeutic indices (LD10/ED90) (*36*), obtained on a number of experimental tumors (table 6), shows that these indices cannot serve as a basis for decisively characterizing the compounds. Depending on the tumor in which the preparation is studied, *one* must be preferred, although the high value of both is well known.

Calculation of like indices (LD10/ED60) from adenocarcinoma 755 for two other widely used antitumor compounds, 6-mercaptopurine and 5-FU, disclosed an indisputable advantage of the former over the latter (12.8 and 0.87, respectively). Both compounds occupy a definite place in human tumor chemotherapy without competing with one another. The chemotherapeutic indices, as L. F. Larionov (*43*) and G. L. Zhdanov (*29*) have pointed out already, are only a more distinct reflection of the selectivity of action of a compound on a specific tumor and, in combination, are a reflection of the spectrum of antitumor effect of the compound. A higher or lower chemotherapeutic index of a compound toward some specific tumor cannot be considered in itself to be either an advantage or a shortcoming. Such indices also have great value if they reflect the effect of compounds on a model system for a specific human tumor. Then comparison of the activity of two or more compounds, of which one is known to affect the process modeled, makes sense, since it assists in selecting the drug deserving the greatest attention. A comparative study of selectivity of an antitumor effect is also important in testing compounds having identical spectra of antitumor action. This makes it possible to establish which drug causes less damage to normal tissues and organs; e.g., asalin, having an identical spectrum of action to that of sarcolysin, was selected (*78*). We believe that the problem of experimental study of selectivity of antitumor action and the criteria for estimation require further study.

Of those compounds selected in the initial tests, certain questions of their mechanism of action are investigated in depth. At IECO, the effect of compounds on cells in various stages of the mitotic cycle (*37*), the dependence of antitumor and toxic effects on time of administration of the compound during the day (*51, 52*) and on hormonal status (*53–55*), distribution of the compounds in tumor-bearing animals, the effect of various factors on this distribution (*53, 57, 58, 77*), and the morphology of treated tumors are being studied in detail (*49, 69–71*). If a compound has any peculiarities of action, special attention is given to them, e.g., the hormone activity of the hormonocytostatic group (*79*). The phyto-

TABLE 6.—*Chemotherapeutic indices of sarcolysin and cyclophosphan (once daily, 10-time administration)*

Drug	LD10/ED90 for:			
	Walker's carcinosarcoma	Sarcoma 45	Schvets tumor	Sarcoma 298
Sarcolysin	12	1.8	7.7	2.4
Cyclophosphan	1.2	1.6	18.7	2.9

hemagglutinins chanerol and chanerosan were also examined in detail (*73*).

The general scheme of in-depth study of antitumor compounds, represented here by the example of IECO, is fundamentally the same in all our laboratories performing experimental tumor chemotherapy; it differs only in details, depending on the specific conditions and tasks a given department is assigned.

Finally, prospects of development of methods of experimental tumor chemotherapy should be emphasized. It is indisputable that the general successes of theoretical oncology are yielding new directions to chemotherapists in the synthesis of antitumor compounds and, simultaneously, new methods of testing them. Perhaps biochemical, virologic, and immunologic methods of research will be used to a greater extent than previously. One can expect here that the number of experiments conducted in vitro in drug selection will gradually increase. It is hardly possible to eliminate in vivo tests. However, the latter apparently will be reserved for solutions to more complicated problems.

Precise mathematical methods have been introduced in tumor chemotherapy (*20, 61, 75, 90, 94, 109*). Use of these procedures in chemotherapy, as in other fields, provided a transition to a qualitatively new level of research; of course, it is difficult now to foresee what specific results this may produce, and only the immediate possibilities are contemplated. Apparently, mathematical methods in the field of experimental tumor chemotherapy can assist in selection of models for testing potential antitumor compounds and in the development of optimum schedules of treatment of specific tumors by one drug or a combination of compounds. They can assist in objective evaluation of each substance and in comparison of compounds by any of the assigned parameters. Depending on the specific conditions, these methods assist in optimization of treatment in forecasting the effect. Beyond a doubt, the process of testing compounds will proceed more rapidly and be less expensive than presently. Ultimately, certain general patterns can be successfully established by mathematical methods, which will assist in working out the correct tactics in searching for and creating compounds with given properties.

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Preclinical Study of Antitumor Compounds

A. B. Syrkin[1]

After new substances have been selected for clinical testing by assays on tumor-bearing animals, they undergo additional comprehensive preclinical study, toxicologically and pharmacologically. Researchers performing the toxicologic aspect have two objectives: 1) to determine if a new compound can be tested on man, and 2) to discover what side effects, if any, result from use of a new drug. Those scientists working on the pharmacologic assays deal with routes of administration, effects of various combinations of drugs, regimens, pharmacokinetics of the compound(s) in the body, and with the spectrum of antitumor activity. This type of separation of the investigations is an arbitrary one, inasmuch as the results of one aspect overlap the other; e.g., in seeking optimum conditions for administration of the drug, new manifestations of toxicity are found.

TOXICOLOGIC INVESTIGATIONS

Tests of all potential new drugs under clinical conditions, including antitumor compounds, are conducted after approval of the Pharmacological Committee, U.S.S.R. Ministry of Public Health, has been granted. In conformance with the requirements of the Pharmacological Committee (*24*), the parameters of the acute (single administration) and chronic (multiple administrations) toxicity are determined on at least three kinds of animals, one of which should be a dog. Attention is paid to the general well-being of the animals, change in body weight, peripheral blood picture, and functions of the liver, kidneys, and cardiovascular system (EKG data). All animals that died during the testing are autopsied. Surviving animals, killed at different times after they had received all prescribed compounds, are also necropsied. Depending on the nature of the compound studied, other examinations are performed, for example, determinations of pyrogenic and anaphylactogenic properties and local irritants. Study of carcinogenic and teratogenic properties of antitumor compounds is not considered obligatory.

Toxicity Parameters

At the U.S.S.R. Academy of Medical Sciences Institute of Experimental and Clinical Oncology (IECO) in Moscow, the following scheme of preclinical toxicologic investigation of new antitumor compounds has been adopted; mice, rats, and beagles are used.

In mice, the acute and chronic toxicity parameters [dose lethal to 10% of animals (LD10), mean lethal dose (LD50), and others], effect of the compound on body weight, and general state of the animals (behavioral reactions) are determined.

In addition to those tests performed on mice, the rats' blood and urine are analyzed biochemically. Electrocardiograms are taken after the rats have been given various doses of the test compound. Some animals given a course of treatment (10–20 administrations) are killed after 1 month and others after they received the course dose. All organs and tissues are examined histologically.

Three dose levels by single and course administration are determined with dogs: lethal, which is obvious; toxic, though not fatal does result in severe intoxication; and tolerable, which results in comparatively light and reversible manifestations. During the tests, the general well-being of the animals is carefully monitored (behavior, body weight, temperature, food intake) and blood and urine are analyzed. The animals are observed for 3 months; then they are killed and necropsied. Any dogs that die during the tests are also autopsied.

The toxicologic investigation protocol described is a general scheme; however, individual compounds by virtue of their specific biologic reactions are studied in additional tests involving different methods of examination.

Study of the effect of new antitumor compounds on animals naturally does not stop completely during the time the preparation is sent for clinical testing. Cooperative work of the clinician-chemotherapists and toxicologists continues during periods I and II of the clinical test phase and later. The necessity frequently arises for a return to previously studied compounds for a more precise definition of some toxicologic reaction or for comparative toxicologic investigations with new substances having antitumor activity and recommended for clinical testing.

A great amount of material has been accumulated that characterizes the toxicity and general effect of antitumor compounds on experimental animals. Substances having antitumor activity and belonging to different classes by chemical structure, as the data indicate, have certain common traits in their reactions on the body. In determination of toxicity of antitumor agents, it is continually observed that death of the animals does not occur immediately after administration of the compound, but after several days. As a rule, single doses approximating LD30 cause death after 2–6 days and later. Only the use of superlethal doses, with damage to the central nervous system, can reduce the interval between administration of the preparation and death of the animal (right up to a few minutes).

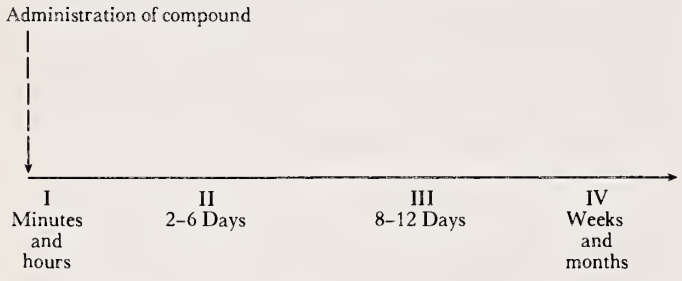
Delayed death of animals after they are given lethal doses is characteristic of antitumor compounds, and it distinguishes them from many other chemotherapeutic and pharmacologic agents. The fact of delayed death after use of some substance can serve as the first and approximate indication of its possible cytotoxic and antitumor activity.

For antitumor compounds, delayed death of the animals is explained by damage to the proliferative capabilities of the organs and tissues (damage predominantly to three systems: gastrointestinal tract, hematopoiesis, and the immune system is typical). However, adult and functioning cells present at the moment of administration of the compound compensate for the

[1] Head, Laboratory of Pharmacology and Zooclinic, Cancer Research Center, Moscow, U.S.S.R.

disruption for a certain time. The singularities of the biologic effects of the compounds determine which of the systems indicated will be the leading ones in the pattern of poisoning. Compounds differing in chemical structure can differ noticeably in the systems they affect. For example, the mucosa of the gastrointestinal tract is most vulnerable to the actinomycins, and a single administration of compounds of this group causes death of the animals in 2–6 days ("enteric" form). For many alkylating agents and antimetabolites, the enteric form of death also is characteristic. However, with hematopoiesis disrupted in addition, these preparations can cause death not only in the first days after administration of the compound but also in 8–12 days ("bone marrow" form). An example of a specific biologic effect of an individual compound is a persistent suppression of immunity (e.g., by cyclophosphan). After this type of compound is used, the animals surviving the enteric and bone marrow near-death periods can die later ("immunodepressive" form) (text-fig. 1). Determination of the times of death is one of the common indicators characterizing the singularities of the biologic effects of the compound being studied. Determination of the toxicity parameters of the compounds for different species of animals helps to ascertain the tolerable dose in man.

The new antitumor compound dizaet (now in the first phase of clinical tests) has been studied (*20*) in four species of animals, with various modes of administration (table 1). Tolerance to the compound increases in proportion to longer intervals between administrations; this reflects the high cumulative capacity of the agent. Recalculation of the dose per square meter of body surface of the animals has shown that doses adequate in toxicity per milligram/square meter for rabbits and dogs are almost the same, but they differ appreciably from those doses for small animals. In selecting the first doses for man, one proceeds from the data obtained in the animal that is most sensitive to the compound.



TEXT-FIGURE 1.—Typical forms and times of death of animals after administration of antitumor compounds. I=death from affliction of nervous system; II=enteric; III=bone marrow; and IV=immunodepressive.

Side Effects

In addition to determination of the toxicity parameters of new compounds, side effects are discovered that require more thorough study. Let us dwell on the principal results obtained under this plan in recent years.

A side effect on hematopoiesis is characteristic of most modern antitumor compounds. In addition, even with profound disruptions of hematopoiesis caused by administration of massive doses of cytostatic preparations to animals (*10*), a high functional activity of the bone marrow reticular cells, initially displaying a tendency toward blast formation, is preserved. Erythronormoblasts and myeloid elements form even with general suppression of hematopoiesis. Consequently, the hematopoietic organs have a high potential for regeneration. Individual antitumor compounds have a high tropism for blood-forming tissues. Thus the effect of carminomycin, the new antitumor antibiotic of the anthracycline series, on blood cell formation in dogs has been studied (*8*). Quantitative changes in the myelokaryocytes and peripheral blood indicators were determined dynamically. Research demonstrated that the antibiotic has a lymphotrophic effect, causing thinning of the lymphoid tissues of the follicles of the spleen and lymph nodes. Death of the animals occurs from aplasia of the bone marrow and most likely infection due to suppressed hematopoiesis.

Dogs are good models to study the reactions of the compounds in the gastrointestinal tract (loss of appetite, vomiting, diarrhea). Morphologic changes are also evaluated on dogs and small animals. In tests on rats, the morphologic symptoms of lesions of the intestinal mucosa were detected not only after administration of intolerable doses but also after a single maximum tolerable dose (MTD) or a course of therapeutic doses (*28*). Thus sarcolysin, thio-TEPA, 5-fluorouracil (5-FU), or 6-mercaptopurine, in the usual therapeutic test doses, caused foci of decomposition of the epithelial cells, atrophy of the villi, and focal atrophic changes in the mucous membranes of the colon. Considerable dilation of the lymph vessels and edema of the stroma of the mucous membranes were observed. The edema aggravated the seriousness of the lesions and hampered regeneration. Severe alterations in the gastrointestinal tract were caused by 5-FU in assays in which paired rats were fed a specific diet. A decrease in food intake because of loss of appetite resulted in a 28% reduction in food utilization, a 17% lag

TABLE 1.—*Toxicity indices of dizaet (1,2-bis-diazoacetylethane) on experimental animals*

Animal species	Administrations			Interval between administrations, hr	LD50, mg/kg	Maximum tolerable dose (MTD, LD10)	
	Mode	Route	Number			mg/kg	mg/m²
Mice	Repeated	ip	7	24	1.3	0.5	1.6
	Repeated	ip	7	72	90.0	75.0	
	Repeated	sc	7	48	3.0	1.0	
	Single	ip	1	—	825.0	600.0	
Rats	Repeated	ip	14	24	0.6	0.1	
	Repeated	ip	7	24	3.0	1.6	10.6
	Single	ip	1	—	150.0	100.0	
Rabbits	Repeated	iv	7	24	15.0	5.0	0.5
Dogs	Repeated	iv	5-7	24	—	2.5	56.8

in weight gain, and 30–40% inhibited growth in individual organs (*18*).

The effect of antitumor compounds on liver function is evaluated experimentally by various methods (radiometrically, by sulfobromophthalein sodium excretion, etc.). Dynamic observations of liver function can be done on small animals (mice, rats) according to the method of Plaa and his colleagues (*29*), and by determination of the length of time the animal remains under the influence of Nembutal (or sodium barbiturate). This method is preferred in toxicologic study of antitumor compounds in long-term experiments (*6*).

In numerous kidney function tests, various alkylating agents (*16*) and the antibiotics olivomycin, rubomycin, and sibiromycin generally gave identical results. Use of the compounds in tolerable therapeutic doses does not significantly affect kidney function, but toxic doses can lead to disturbances which ultimately result in death of the animal (e.g., sibiromycin administration).

The pathways of the cardiovascular and respiratory systems can be followed by short-term tests on rabbits or cats, in which the carotid artery is connected (through a cannula) with a manometer, and the arterial pressure and pulse rate are recorded, as are depth and rate of respiration. In long-term tests, the carotid arteries of rabbits are led out into a skin flap, which allows one to determine the animal's blood pressure while the antitumor compound is being given. Electrocardiograms are also taken of rats, rabbits, and dogs. The physiologic examinations are supplemented by morphologic study of the tissues. Thus after rubomycin is given to animals, any damage to the pericardium (focal hemorrhage and edema) and the right auricle (hemorrhage in the epicardium and dystrophic changes of the myocardium) can be revealed (*4*). A similar pattern was noted in the investigation of sibiromycin (*14*).

Carminomycin, in single and multiple iv injections given to dogs at toxic and lethal doses, did not significantly affect the electrocardiographic indices (*8*).

Since one of the principal side effects of antitumor compounds is suppression of immunity, preclinical studies of new substances must be oriented toward this problem. Considering that the tumor process itself frequently is immunosuppressive, animals with tumors are suitable models. Complex interactions may be observed during chemotherapy when the immunity of tumor-bearing animals is being determined. In particular, transplantation of an ascites or solid tumor leads to a sharp reduction in immunologic reactions. Chemotherapy with rubomycin C displays two opposite effects: It suppresses immunogenesis and inhibits development of the blastoma process. Because of the inhibitory effect, the reduction in immunity under the influence of the tumor is retarded. Nevertheless, immune reactions are decreased in treated animals (*27*).

The dose level of antitumor compounds is most important. An increase in dose is accompanied by greater therapeutic effect up to a certain threshold, after which still higher doses of the compound can cause persistent suppression of antitumor immunity, which in turn leads to metastases and death of the animals. Thus after various alkylating agents (sarcolysin, asalin, asaley, cyclophosphan, embichin) were given in doses close to the maximum tolerable one, 86 of 126 rats with tumors (sarcoma 45, Walker's carcinosarcoma) died. Metastases in the lungs were found in 25 of them (29.1%) and were also observed in the lungs of some of the rats after complete resorption of the primary tumor. The corresponding tumor was repeatedly transplanted successfully in 46.7% of the surviving animals 1–3 months after resorption of the primary lesion.

Another pattern was revealed when optimum doses of the same compounds, amounting to one-half to one-third of the maximum tolerable ones were given. Of 80 rats treated in this manner, 14 died. Metastases were found in the lungs in only 1 rat (7.1%) of the 14. Of 99 corresponding control animals, 90 died, and metastases also were found in the lungs of only 1 rat. The use of optimum doses led to a persistent therapeutic effect, and repeat transplants succeeded in only 4.3% of the cases. In control rats of the same age as the test animals, tumors were transplanted in 92% of them. It is interesting to note that, after treatment with cyclophosphan, which has a pronounced immunodepressive effect, development of resistance to repeated tumor transplants in cured animals was not observed. Analysis of data on side reactions in the literature (*22*) assisted in determination of those side effects. With corresponding experimental approaches, side effects of compounds were determined on hematopoiesis, liver, kidneys, gastrointestinal tract, cardiovascular system, mucous membranes (bladder), lungs, endocrine glands, immune systems, and development of fetuses.

PHARMACOLOGIC INVESTIGATIONS

The development of optimum conditions for therapeutic activity of compounds to be manifested begins before clinical tests are initiated; however, this aspect of the investigation frequently continues for a long time, and for those compounds used in medical practice, ways are sought to improve their effectiveness. Evaluation of their activity by administration by different routes is important, not only in selection of the most efficient method under clinical conditions, but also for information on the fate of the compound in the body. A comparative investigation of antitumor compounds is one of the most important tasks of pharmacologists in research.

By the ip route, a significant amount of the preparation enters the portal blood circulation and passes through the liver barrier, where it can be inactivated. In approximating experimental conditions to those of the clinic, compounds must be administered by the same method as will be used for a patient with cancer.

Studies (*13*) with *N*-acetylsarcolysin peptides (asalin, asaley, asaphan) showed that these compounds have similar effectiveness in adequate toxic doses administered to the stomach im, or rectally. However, it was discovered that dystrophic changes of the tissue develop at the im injection site. Tests of suspensions, suppositories, and aerosols for rectal use indicated these vehicles might serve as means to administer *N*-acetylsarcolysin peptides.

Regional administration of compounds deserves attention. In tests on rats with sarcoma 45, intra-arterial regional application of cyclophosphan and the Romanian preparation IOB-82 was used. For this purpose, the tumor was implanted in the muscles of the lower third of the femur of rats and a single intra-arterial infusion of the compounds was injected after 1 week. The external iliac artery was released surgically under anesthesia, and a solution of the preparation was injected and then the artery was ligated. The intra-arterial administrations were regional with respect to the tumor, or nonregional, when the injection was made in the footpad opposite the one in which the tumor was growing. In preliminary tests, the procedure of intra-arterial injection did not significantly affect the growth rate of these tumors.

Combined summary data, obtained in assays on rats with sarcoma 45 and given cyclophosphan over a large dose range, are presented in table 2: 11 doses of 600–9,375 mg/kg in the stomach; 6 iv of 12.5–400 mg/kg; and 6 doses of 12.5–400 mg/kg intra-arterially.

TABLE 2.—*Toxicity indices and effects of cyclophosphan on tumor and hematopoiesis*

Indices[a]	Route of administration			
	Stomach	iv	Intra-arterial	
			Regional	Nonregional
LD10 (MTD), mg/kg	140	100	100	100
ED-L50, mg/kg	18	38	75	76
ED-T50, mg/kg	128	88	78	47
I_1 = LD10/ED-T90	1.1	1.1	2.1	1.3
I_2 = ED-L50/ED-T90	0.1	0.4	1.0	1.6

[a] *See* text.

TABLE 3.—*Comparative toxicity of carminomycin and rubomycin by various methods of administration to 18- to 20-g white mice*[a]

Route of administration	LD50, mg/kg	
	Carminomycin	Rubomycin
iv	3.7 (3.4–4.0)	26.1 (19.0–35.7)
ip	1.3 (0.9–1.9)	5.5 (4.5–6.6)
sc	3.8 (3.2–4.6)	40.9 (37.8–44.2)
Peroral	7.5 (4.8–11.6)	205 (169–348)

[a] *See (11).*

The toxicity of the preparation, as median lethal doses (LD50), differed little by the various routes of administration. LD50 doses were practically identical iv and intra-arterially; it was approximately one-third higher only by administration of an adequate dose to the stomach. The MTD (LD10) were similar by enteral and parenteral administration. The ED-L50 (doses causing 50% reduction in number of leukocytes) were practically equal by regional and nonregional administration; however, the ED-T90 (dose leading to 90% inhibition of tumor growth) was 1.6 times less by the regional route to the artery. Consequently, in both instances of intra-arterial administration, the manifestation of a toxic effect on leukopoiesis, upon reaching identical levels of effect on the tumor, is significantly less by the regional route. It is evident from the therapeutic index (I_1) that the antitumor effect of cyclophosphan is the same by enteral or parenteral regional administration. The therapeutic index in regional arterial administration is twice as high. This is evidence of a greater selectivity of the effect of the compound by this method of administration. The I_2 index (ratio of the ED-L50 and ED-T90 doses) is distinguished in regional intra-arterial administration in comparison with other routes. According to the I_2 index, there also are conditions that guarantee a greater effect of the drug on the tumor, with an equal effect on hematopoiesis, by intra-arterial nonregional administration than by the stomach or iv.

The comparative effectiveness of the Romanian preparation IOB-82 was studied by the method described, and it was determined that none of the routes of administration investigated has a significant advantage.

A comparative investigation (*11*) of the toxicity of various routes demonstrated (table 3) that carminomycin is four times more toxic ip than rubomycin, seven times iv, 11 times sc, and 27 times perorally. Consequently, a slight difference in the structure of the rubomycin and carminomycin aglycone leads to an increase in the toxic properties of carminomycin (expressed particularly when it is given perorally) and indicates better absorbability of carminomycin than rubomycin in the gastrointestinal tract (*11*). Carminomycin also has more pronounced cumulative properties than rubomycin, which must be taken into account when use of this antibiotic is planned.

There is no necessity for proving the importance of development of schemes for administration of antitumor compounds. Tests (*17*) with the well-known preparation sarcolysin verte demonstrated a better result (more cured mice) with a 24-hour interval between two injections of sarcolysin. The effect was twice that of a single administration at a double dose or by two injections with different intervals between them. It is important to note that intervals between injections such as 48, 72, and 96 hours reduced the effectiveness of sarcolysin.

In tests on another more rapidly growing tumor (*25*), sarcolysin was more effective with a 72-hour interval between injections. For tumors with different growth rates, optimum schemes of drug use may differ. Consequently, in the preclinical stage of study, there is interest in developing optimum schemes for use of a new preparation on tumors with different growth rates.

For most modern antitumor compounds, the property of growth suppression and tissue regeneration is characteristic. Investigation of the effect of antitumor compounds on growth and regeneration of normal tissues is interesting mainly from the toxicologic point of view. This kind of investigation provides additional knowledge for evaluation of the damage compounds have on various normal tissues. Some observations indicate a probable similarity in reactions of tumors and the corresponding normal tissues. Thus corticosteroids (prednisone) used for treatment of lymphoid leukemia were proposed because of their lymphopenic effect in animals and man. Discovery of the therapeutic effect of embichin (U.S.A.) and novembichin (U.S.S.R.) in hemoblastosis was related to observations of the effect of these agents on hematopoiesis. Myleran was proposed for treatment of myeloid leukemia because of its preferential action on granulocytopoiesis. The preparation DDD has been used in tumors of the adrenals, since it causes selective atrophy of the adrenal cortex. The examples enumerated, the number of which can be increased, allows one to propose that the spectrum of antitumor activity of a new compound being studied can be predicted from the extent of its preferential action on growth of specific tissues and determination of its organ-directed effects. For example, the preferential effect of bruneomycin on the lymphoid system that was revealed experimentally was the basis for the first tests of the compound on patients with malignant lesions of the lymph glands. The results confirmed the proposal based on experimental data (*26*); lymphoid-type tumors are sensitive to the antibiotic.

Various approaches are used for revealing organotropism of new antitumor compounds: evaluation of weight change (wet and dry) of growing animals (*19*), determination of the effect of compounds on physiologic regeneration of hematopoietic tissues, evaluation of the action of preparations on reparative generation of the liver (*21*), ovaries (*1*), and other organs.

One of the problems encountered in pharmacologic investigation of antitumor compounds is their fate in the body, i.e., characteristics of entry, distribution, transformation, and elimination. This area of the investigation, designated "pharmacodynamics," can give information that facilitates development of optimum conditions for administration of the prepara-

tion, data on its tropism, and the extent of its accumulation in tumor tissues.

Three principal methods are used for quantitative determination of any drug in body tissues and fluids, depending on the singularities of the compound: biochemical, radiometric, and biologic.

Spectrophotometry is used for indication of alkylating agents, e.g., the ability of alkylating agents to form chromophore groups with 4-γ-(nitrobenzyl)-pyridine (*15*), because the spectrofluorometric method is a highly sensitive one.

The radiometric method of examination is widely used in the study of the pharmacodynamics of antitumor compounds, such as fluorafur. Comparative studies of fluorafur and 5-FU revealed a definite difference in the pharmacodynamics of these two structurally similar compounds (*9*).

The biologic approach also deserves attention. Many antitumor compounds can be identified by microbiologic methods.

Moreover, determination of the time an antitumor compound remains in the blood can be made from the affliction of sensitive organs or tumors, to which the blood flow was stopped at various times after administration of the substance. The simplest way to accomplish this is to apply a tourniquet to the limb of an animal and then count the number of karyocytes in the bone marrow of the "protected" and "unprotected" limbs.

A FINAL WORD

A many-sided preclinical investigation of the toxicologic and pharmacologic properties of new compounds requires the expenditure of considerable time and resources. Practical experience in this area shows that the efforts expended are not in vain. Comprehensive experimental study of the singularities of biologic action facilitates the development of optimum use of new drugs in the clinic.

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Clinical Testing of Drugs for Cancer in the United States and the Soviet Union

James F. Holland[1]

In the United States and in the Soviet Union, deliberate developmental programs for cancer chemotherapeutic drugs are being conducted. Several differences and similarities have characterized these two efforts.

In the United States, an overall supervisory role is centered in the National Cancer Institute (NCI), and governmental employees of that body are charged with familiarizing themselves with available compounds from all over the world (particularly from American sources) and with bringing promising candidate compounds to clinical assessment. This is done by eclectic solicitation for testing in governmentally organized laboratories of drugs from many sources, which may include private and state universities, the pharmaceutical industry, laboratories supported by government contracts, and also compounds submitted from chemical companies and those obtained from international contacts.

The pharmaceutical industry and, on a smaller scale, nongovernmental and university laboratories test compounds for possible anticancer effects in various preclinical screens. Much of the preclinical testing is performed at nongovernmental laboratories and research organizations that are under contract.

In addition to the federal employees administering this program, some individuals in private and state universities as well as in the pharmaceutical industry devote their personal attention exclusively to evaluation of new compounds.

Compounds shown to have activity in preclinical systems are for the most part channelled into the federal organizational structure of the NCI for primary testing in man. Compounds, whatever their source, must be submitted to the Food and Drug Administration (FDA) before any clinical tests can be performed. This federal agency surveys the information available on toxicology and pharmacology to ascertain that all drugs entering clinical trial have been appropriately studied to assure patient protection. The FDA also is charged with the responsibility of reviewing clinical protocols; patient safety is the primary concern. In effect, the FDA has delegated some of this responsibility to the NCI for drugs that the Institute sponsors for clinical trials.

When a private sponsor or the NCI has approval from the FDA to investigate a new drug in man, initial clinical trial for toxicology (phase I) begins. This first investigation may take place in hospital facilities of the NCI itself (these are located in Bethesda; Washington, D.C; or Baltimore) or in a nongovernmental institution. This latter type ordinarily undertakes such an investigation based on the competence and interest of its staff. Two state cancer institutes and one private, and about six universities have conducted most of the nongovernmental phase I studies with cancer chemotherapeutic drugs.

Committees of investigators within American institutions are established by law to review the research programs and experimental designs proposed for new drug studies within their institutions. They are responsible for ascertaining that unnecessary risks are avoided. The protocol for study must be detailed and specific with respect to patient selection, tumor types, procedures to be undertaken at specific times, criteria for evaluation, and techniques of dose escalation. The objective is to define an appropriate dose for eventual clinical trial. Substantial attention has been paid in recent years to variations in dose scheduling. After a single or a daily dose tolerance has been established, drug tolerance is usually sought to other regimens, to repeated doses, and particularly to short courses of drugs such as might be given over periods of 5 days or a week.

During initial studies of drugs, repeated examinations of the patient and of his functional and biochemical integrity are conducted. Study of drug concentrations in biologic fluids is undertaken in some institutions so that pharmacologic characteristics of dose, rate and route of administration, and scheduling are better understood. These studies often lead to a second stage of pharmacologic investigation, i.e., the synthesis of isotopically labeled drug and the study of radioactive species in biologic fluids.

Ordinarily, when an acceptable drug schedule is found, there is considerable interest in seeking evidence of any antitumor effect that may have occurred in this initial group of patients. Despite failure to see any positive antitumor activity in this initial group, the commitment is almost invariably designed to evaluate activity in a survey of patients with advanced cancers (phase II study). If the preclinical data were promising enough for phase I study, the only deterrent to initiating a phase II study would be the possibility of encountering hazardous toxicity (such as cardiac arrhythmia, central nervous system toxicity, or other acute and unpredictable side effects) that would make exploration for antitumor effects unwise.

A spectrum of patients with various types of advanced cancer is selected for phase II study. Often this is done in the same institution where the phase I study was conducted, but frequently, several institutions will collaborate.

There are about 20 groups in the United States, each containing from 10 to 30 member institutions. Each group is headed by a chairman, who ordinarily is not a government employee. The association within these groups is voluntary and related to their common interest in cancer treatment. Their financial support is derived from government contracts or grants; participation is not obligatory. Most group-participating institutions are not cancer specialty institutes but are general hospitals where patient care is rendered and other research is in progress.

[1] Chairman, Department of Oncological Diseases, Mt. Sinai School of Medicine, New York, N.Y. 10029.

Information from these research studies is returned at 3- or 4-month intervals to NCI, where long-range strategy is formulated, drug requirements are calculated, and drug purchase or synthesis is authorized. An assessment is made of antitumor activity, and primary information is exchanged with other groups for subsequent studies.

In the Soviet Union, a supervisory role is partially centered in the Institute for Experimental and Clinical Oncology (IECO), of the Academy of Medical Sciences in Moscow, where the All-Union Chemotherapy Center is located. This Center is responsible for procurement of drugs through the Ministry of Health and for conduct of studies and of clinical pharmacologic trials. Drugs are acquired from government laboratories and from the cancer research institutes of the several Republics. There are more cancer institutes but fewer universities involved in cancer research in the Soviet Union than in the United States.

There are approximately eight laboratories for large-scale animal tumor screening in the Soviet Union. Drugs synthesized or isolated in other institutions or laboratories are made known to the IECO. Although preliminary animal testing may go on in the originating institution, pharmacologic and preclinical toxicologic studies are usually performed at IECO, as are any primary clinical trials.

In the Soviet Union, there is a committee of approximately 50 physicians and pharmacologists, subcommittees of which (on an ad hoc consultative basis) decide the appropriateness of undertaking studies with different drugs. Thus an ad hoc panel might be empowered to decide whether studies with a particular new drug should be undertaken in much the same way as the continuing staff of the FDA would make similar determinations in the United States.

The initial drug study is ordinarily started on the Clinical Pharmacological Service at IECO. The dose is escalated systematically until an appropriate level is found at which some host and/or tumor effect is seen. It is then forwarded to the Clinical Chemotherapy Service, IECO, which undertakes experimental therapy with the drug in patients; various tumors are selected for treatment. Clinical trials often are run with single agents or with combinations of two or more drugs on approximately 50 patients. Cooperative multi-institution groups have not been widespread in the U.S.S.R. Thus many of the other institutes and cancer hospitals use drug doses, schedules, and combinations that have been devised and stipulated by IECO and to a lesser extent by the Petrov Institute in Leningrad, which is also authorized to undertake phase I and phase II studies. No other Soviet institutions participate in initial clinical toxicologic and activity trials on cancer chemotherapeutic compounds.

Cooperative programs with surgery and chemotherapy or combination chemotherapy have been studied in hospitals chosen by the All-Union Center. They are selected because of their particular patient populations and because of their effectiveness and skills. All plans for study are formulated in the All-Union Center.

Drugs and protocols are also supplied by the All-Union Center. Data are returned from the several institutes to the All-Union Center for analysis and promulgation. Meetings for operational updating and exchange of views among the participating institutions are held in association with other annual meetings.

Final decisions on the purchase of foreign drugs or the manufacture of drugs are not invested in IECO but rather in the Ministry of Health.

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